AN ABSTRACT OF THE THESIS OF

Marion S. Murray for the degree of Master of Science in Plant Pathology presented on April 10, 1995. Title: Susceptibility of Pacific Yew (*Taxus brevifolia* Nutt.) to *Phytophthora lateralis*

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In 1991 Pacific yew (*Taxus brevifolia* Nutt.) was reported as a new host for *Phytophthora lateralis* Tucker and Milbrath which is an aggressive root rot pathogen thought previously to be specific to Port-Orford-cedar. This study was designed to compare the pathogenicity of *P. lateralis* on the two hosts, and to characterize sites where Pacific yew mortality occurs. The specific objectives were: 1) compare root colonization and mortality of Pacific yew and Port-Orford-cedar seedlings and rooted cuttings; 2) compare lesion length on inoculated Pacific yew and Port-Orford-cedar branches and stems; 3) compare zoospore attraction to freshly cut Pacific yew and Port-Orford-cedar rootlets; 4) compare amount of mortality of Pacific yew and Port-Orford-cedar in infested drainages and determine extent of yew mortality; and 5) characterize sites where *P. lateralis* causes Pacific yew mortality.

Root colonization of *P. lateralis* was significantly greater in cedar than in yew. Seedling mortality averaged 58% for cedar and 4% for yew. Lesion length on the cedar seedling stems was twice the lesion length on yew stems, and cedar branches had lesions four times longer than yew branches. Abundant zoospore aggregation occurred on cedar rootlets along the zone of elongation and the region of maturation. In comparison, far fewer zoospores encysted along the yew rootlets, and were concentrated on the root hairs. The stream survey of three infested drainages in southwest Oregon and northwest California revealed a total of 1199 dead Port-Orford-cedar (46% mortality), and 86 dead Pacific yew (10% mortality). At sites where *P. lateralis*-induced mortality occurred, the interaction of slope and distance from the stream was negatively correlated with tree death.

Based on results of this study, we conclude that Pacific yew is less susceptible to *P. lateralis* than Port-Orford-cedar, but that mortality of Pacific yew in the field is greater than previously reported. In addition, Pacific yew mortality was observed most often on level to nearly-level sites close to the stream's edge where root exposure to *P. lateralis*-infested water was frequent in scope and duration.

Susceptibility of Pacific Yew (*Taxus brevifolia* Nutt.) to *Phytophthora lateralis*

by

Marion S. Murray

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

Susceptibility of Pacific Yew (*Taxus brevifolia* Nutt.) to *Phytophthora lateralis*

Chapter 1. Introduction and Literature Review

INTRODUCTION

This study is the first in-depth examination of the susceptibility of Pacific yew (*Taxus brevifolia* Nutt.) to *Phytophthora lateralis* Tucker and Milbrath, a root rot pathogen initially reported on the host in 1991 (DeNitto and Kliejunas). The relative susceptibility of Pacific yew was compared to the primary host of the pathogen, Port-Orford-cedar (*Chamaecyparis lawsoniana* (A. Murr.) Parl.) through root, stem, and branch inoculations, and through a field survey of infected drainages. In addition, a plot study in southwest Oregon and northwest California was performed to determine the most important environmental factors associated with mortality of Pacific yew.

Pacific yew, in the family Taxaceae, is not an economically valuable tree species aside from the use of its wood for specialty items such as tools, hunting, and fishing implements (Hartzell 1991). Pacific yew is native to a large portion of the Pacific Northwest and regions of the Rocky Mountains. It occurs on all soil types, in several different vegetation classifications, and at various elevational zones. Pacific yew is a small understory species that is usually a minor component of the forest stand. It is important ecologically for stream channel stabilization and for stream shading. Conversely, Port-Orford-cedar, in the family Cuppressaceae, is an extremely valuable species; stumpage prices have reached into the thousands of dollars per thousand board foot. It is native to a small, floristically unique region of the Pacific Northwest, in northern California and southern Oregon. Although it can be found on dry serpentine slopes, it is most commonly associated with riparian and other wet areas. In localized areas, Port-Orford-cedar can be the dominant or sometimes the only tree species that occurs.

In the region of Port-Orford-cedar, and in particular northern California where the soils are drier, Pacific yew is most concentrated in the Port-Orford-cedar series, in the wet valley bottoms and riparian areas. It is in these wet areas where *P. lateralis* thrives and easily spreads. Heavy mortality of Port-Orford-cedar has resulted in ecological disturbance and economic losses. Resistance in Port-Orford cedar is very low, and as such, almost complete mortality has occurred in some micro-sites. Ecological changes are most severe in areas where the cedar is the dominant tree species, and include modifications in the nutrient cycle, loss of wildlife habitat, an increase in stream temperature, and soil erosion. Economic losses have ranged in the millions of dollars. Loss of Pacific yew appears to be of much lesser importance. The most recent examination of Pacific yew mortality was carried out through a walking survey of major infected drainages. The result was 17 dead yew trees from which *P. lateralis* was successfully isolated (DeNitto, USDA Forest Service, pers. comm.). The effect of Pacific yew mortality in these areas is difficult to determine, but based on the apparent low number of deaths, one can predict that changes in the local environment will be slight.

Phytophthora lateralis can currently can be found in almost all areas of the range of Port-Orford-cedar in southern Oregon and northwest California that are accessible by road or trail. The greatest extent of yew mortality, as determined by the USDA Forest Service survey in 1991, occurred in northern California. All of those trees were located in areas with extensive cedar mortality and in seasonally saturated soils. This most recent report of the pathogen on Pacific yew raises several pertinent questions, such as how susceptible is Pacific yew? Port-Orford-cedar trees often die within a few years of initial infection, however, time of death after infection of Pacific yew is unknown. Also, in infected drainages, where does yew mortality most often occur?

This thesis addresses the above questions through explanation of several experiments and field observations. Following this introduction is a general literature review providing the current state of knowledge of *P. lateralis* and its hosts. The bulk of the review deals with Port-Orford-cedar because, up to 1991, the pathogen was thought to be specific to this host. The second two chapters are designed in manuscript format, each with its own introduction, discussion, and literature cited sections. The first of these deals with the majority of the laboratory work and some field work, while the second covers the remaining laboratory work and a field study. The thesis concludes with a chapter summarizing the pertinent thesis conclusions and addressing future research needs, followed by a complete listing of literature cited.

GENERAL LITERATURE REVIEW

Hosts

Port-Orford-cedar and Pacific yew are the primary hosts of *Phytophthora lateralis*. Many cultivars of Port-Orford-cedar were tested by Tucker and Milbrath (1942) and found to be susceptible. Other *Chamaecyparis* species found to be susceptible, but highly tolerant, include *C. obtusa* (Siebold and Zucc.) Endl. var. *crippsii* and *gracilis*, and *C. nootkatensis* (D. Don) Spach. Port-Orford-cedar is native to a localized area that extends from the Coast Range and northern Siskiyou Mountains in Coos County, Oregon, south to California's northern Coast Range in mid-Humbolt County, and east to outlying populations.

Pacific yew (*Taxus brevifolia* Nutt.) was first reported as a host of *P. lateralis* in 1991 (DeNitto and Kliejunas), and a 1991 U.S. Forest Service (USFS) field survey resulted in successful isolations of *P. lateralis* from 17 dead yew trees (DeNitto, USDA Forest Service, pers. comm.). Preliminary susceptibility tests on Pacific yew were performed by John Kliejunas of the USDA Forest Service, Forest Pest Management in 1991 (pers. comm.). Ten yew cuttings and 10 Port-Orford-cedar seedlings were inoculated with *P. lateralis* through the addition of macerated mycelium into the soil. After 16 weeks, lesions at the root collar were seen on all the Port-Orford-cedar seedlings and on half of the yew cuttings.

The slow growing Pacific yew occurs as a small understory tree and is associated with a variety of conifer and hardwood tree species. The range covers the southern tip of southeast Alaska south to Calavaras County, California. Occurrence of Pacific yew declines in the Coast Range south of the Olympic Peninsula in Washington and north of the Umpqua River in southern Oregon

(Bolsinger and Jaramillo 1990). Stands of yew can be dense and abundant in southern Oregon (Franklin and Dyrness 1972) and in the Northern Rocky Mountains (Daubenmire and Daubenmire 1968). It grows on the western slopes of the Rockies in British Columbia, Idaho, and Montana, where it occurs as a minor, often shrub-like component. On the South Fork of the Clearwater Basin in north-central Idaho, however, Pacific yew becomes a dominant on approximately 16,000 hectares (Crawford and Johnson 1985).

Pacific yew can occur in a variety of environmental conditions, although it grows best in areas with deep, moist, gravelly soils with a rainfall of 80-130 cm/year. It occurs in various plant associations and, over its entire range, occurs most frequently in the understory of Douglas-fir stands (Bolsinger and Jaramillo 1990). In the Coast Range and Klamath Mountains of northern California, however, Pacific yew is most commonly associated with the cool, wet, riparian areas in the Port-Orford-cedar series and least commonly found at mid to upper slopes and/or in the Douglas-fir or white fir/red fir series (Scher and Jimerson 1989). Scher and Jimerson propose that the distribution of Pacific yew in northern California is due to the frequency of stand replacing fires, which occur most commonly at higher elevations (every 33-200 years) and least commonly in riparian areas (every 300-500 years).

Pacific yew is a dioecious species, and birds and mammals are the main vectors in seed dispersal. Seeds require a dormancy period which is only broken by stratification of approximately 20 C at night and 30 C during the day (Bolsinger and Jaramillo 1990). Reproduction of yew can also occur vegetatively, sprouting from cut stumps or rootstocks.

Range of *Phytophthora lateralis*

In 1952, Port-Orford-cedar root rot was first reported near Coos Bay, Oregon, in the northern extent of the cedar's range, possibly introduced through the planting of infected cedar ornamentals (Roth, Trione, and Ruhmann 1957). During the next decade, urbanization and construction spread the fungus south and inland. Today, aside from high elevation stands, *P. lateralis* occurs in almost all locations where Port-Orford-cedar grows, including sporadic reports on ornamentals planted up and down the west coast of the United States and British Columbia.

The disease had not been reported in California before 1980, when Kliejunas and Adams (1981) conducted a survey in northwest California to determine the extent of *P. lateralis*. The fungus was isolated from eight of 50 survey sites, located along the Smith River drainage, and in the town of Eureka, California. The researchers recovered unpublished reports that cedar trees had been dying at one site since the mid-1960s. The remaining sites appeared to have been infested within the last few years. Movement of soil through logging operations seemed to have been the cause of spread on the Smith River sites, while the infestation of the Eureka site may have been due to infected cedar stock.

The range of *P. lateralis* within the Pacific yew populations has not been mapped, but according to the 1991 USFS survey, all the known dead yew trees were located in 11 riparian areas of the Smith River drainage in northwest California, totaling an area not more than 15 ha. All dead yew trees were located in heavily infected stands of Port-Orford-cedar, and near slow moving water or in saturated soils.

Considerations of Pathogen Origin

Due to the epiphytotic nature of the disease, and the fact that natural resistance in the primary host is almost nonexistent, *P. lateralis* is currently accepted as a nonnative species in the United States (Roth, Harvey, and Kliejunas 1987; Roth, Trione, and Ruhmann 1957). Its geographic origin is a question that has yet to be answered. Asia has been hypothesized as a source of introduction due to the fact that Asian *Chamaecyparis* species show high tolerance to *P. lateralis*. The pathogen, however, has never been reported in Japan or Taiwan, two countries with economically important stands of *Chamaecyparis* species.

Another theory is that *P. lateralis* is endemic within the range of Alaska yellow-cedar (*Chamaecyparis nootkatensis*), a tolerant host species. Some believe the fungus could exist in the native range of Alaska yellow-cedar without producing obvious symptoms. Thus, when the ornamental Port-Orford-cedar plantings converged with the native population of Alaska yellow-cedar in southwest British Columbia, the fungus acted as an aggressive pathogen on Port-Orford-cedar, and from there was eventually spread southward. However, *P. lateralis*, has not been reported in Alaska. In addition, the natural range of the two species overlaps in northwest Siskiyou County, CA, where the disease has not yet been reported (Zobel, Roth, and Hawk 1985).

Some have speculated that the discovery of Pacific yew as a new host for this pathogen may mean that perhaps *P. lateralis* is endemic to North America on Pacific yew. A recent study demonstrated significantly reduced mycelial growth of *P. lateralis* in media containing various taxol analogs when compared to controls (Wagner and Flores 1994). The authors proposed that the reason for taxol formation in *Taxus* spp. is for protection against pathogenic Oomycete fungi. Such evidence suggests that Pacific yew may have coevolved with *P. lateralis* resulting in this formulation of defense chemicals.

Pathogen Biology

Phytophthora is one of two genera within the Family Pythiaceae of the order Peronosporales of the class Oomycetes, currently in the Kingdom Protista. Over 40 species of *Phytophthora* have been classified, and *P. lateralis* is differentiated from these by its abundant, lateral, sessile, yellow-brown chlamydospores, among other characters.

Trione (1974) described the sexual stage, and also broadened the description of the asexual stage. Oospores are thick walled, spherical, terminal, and with a diameter of approximately 40 μm. Although the fungus is homothallic, oospores are not always produced in single-strain cultures. Trione observed oospore formation most frequently on agar amended with Port-Orford-cedar foliage. Single spore colonies produced an abundance of oospores, and pairing did not significantly increase the number produced. Germination of the sexual spores was rarely seen, and if so, usually occurred after two months at 5 C, with a single germ tube usually giving rise to a single sporangium.

Unlike many *Phytophthora* species, sporangia of *P. lateralis* usually remain attached to the sporangiophores. Sporangiophores are capable of branching at the base of a sporangium, or growing through an empty sporangium. At the time of release, approximately 25-40 mature zoospores emerge from each sporangium (Trione 1974). According to Englander and Roth (1980), maximum numbers of sporangia can be produced by cultures grown in V8 broth amended with 20 μg/ml β -sitosterol in either full light or 12 hours of light per day at 10-20 C. The temperature and nutritional requirements are typical of the winter months where both host and pathogen occur.

Trione (1974) frequently found chlamydospores in media with high food reserves that were incubated at temperatures of 15-20 C. However, Englander and Roth (1980) found chlamydospore production to be most abundant in V8 broth amended with 20 μg/ml β-sitosterol grown in the dark at 24-25 degrees Celsius. These figures are consistent with Trione's (1959) hypothesis that chlamydospores serve as a mechanism for survival in high temperatures.

Infection Biology

Histological examinations of *P. lateralis* in Port-Orford-cedar have not been previously reported. Several studies, however, give general insight into the infection biology (e.g., Roth, Trione, and Ruhmann 1957; Trione 1959). Motile zoospores, which serve as the primary infection source, emerge from sporangia only in surface water or in saturated soil. These spores percolate into the soil and infect actively growing "humus strivers", which are defined as roots with unlignified tips that grow up into the surface soil and duff (Zobel, Roth and Hawk 1985), or fallen green Port-Orford-cedar foliage.

Zoospores are initially attracted to host tissue by chemotactic means. In a study of the pathogenesis of *P. cinnamomi* on avocado, Zentmeyer (1980) found that zoospores were attracted primarily to the region of elongation, with fewer zoospores aggregating at the root tip and the zone of differentiation. Zoospores encysted at different distances from the root tip, as if in response to a concentration gradient of some stimulatory chemical exuding from the root, such as sugars, amino acids, ethanol, or various cationic molecules. For many *Phytophthora* species, germination occurs within one hour of zoospore encystment. Wounding is not necessary for penetration, which occurs intercellularly through the middle lamellae by enzymatic and mechanical means (Phillips 1993).

Vegetative growth in the forest is limited to living host tissue. Ostrofsky, et al. (1977) showed that mycelium does not occur independently in the soil. Instead, mycelium occurs only in host tissue, invading the phloem of the entire root system. *Phytophthora lateralis* kills tissues as it advances, and once it reaches the host's root collar, the tree is dead, and vegetative mycelial growth usually does not continue. The fate of the vegetative thallus of *P. lateralis* in the tissue once the tree dies is unclear (Trione 1959). Chlamydospores will form, however, in infected root tissue and fallen green foliage during warm, dry weather. As this tissue decays, these resting spores become part of the soil organic matter, and may survive 7 or more years without living host tissue (Hansen and Hamm, in preparation).

As the fungus advances in the host tissue, a margin can be seen under the outer bark where the colonized cinnamon brown necrotic tissue meets healthy cream-colored phloem tissue, most evident on recently killed trees (Roth, Harvey, and Kliejunas 1987; Roth, Trione, and Rhumann 1957; Trione 1959; Tucker and Milbrath 1942). Root symptoms are most obvious in the first two to three years of infection. After the infected tree dies, uninvaded tissue fades to brown and the margin between colonized and uncolonized tissue is less obvious.

At the stage when the roots are fully invaded, a secondary symptom, change in foliage color, is visibly apparent. Due to desiccation from lack of water, foliage wilts and fades in color, from a brilliant green to blue-green to yellow, orange, red, brown, and finally to gray. During the hot, dry summer, foliage symptoms on small trees may progress in a matter of weeks, but, cool, damp weather slows the process down to several months (Trione 1959).

Areas of infection are patchy and vary in size from a few feet to several hundred feet in diameter. Boundaries are hard to discern, as *P. lateralis* does not primarily spread by root grafting. Mortality is associated with areas of water saturated soils, especially in the northern California portion of cedar's range, where the soil is drier and both the host and pathogen depend upon seepage (Gordon and Roth 1973). In the northern range of Port-Orford-cedar, where the soil is more moist, infection centers are correlated with human activity, resulting in a distinctive, scattered, net-like pattern of mortality (Zobel, Roth, and Hawk 1985).

Foliar infection of Port-Orford-cedar can occur during the wet season when low hanging branches touch infested soil or standing water. Trione (1974) showed that the minimum infection time of zoospore-foliage contact is less than two hours. Once infection occurs, fungal growth up the branch can kill lateral twigs and secondary infections on nearby branches may occur by rain splashed spores. Symptoms are noticeable within three days, and include a dull, water-soaked appearance to the foliage, and a color change advancing from gray-green to dull orange-brown to red-brown. If the fungus reaches the bole a canker will form and tree parts above the canker will die (Trione 1974). Infection of aerial foliage of Pacific yew has not been observed.

Fungal Spread

Movement of infected soil and water, and root grafting, are the primary factors involving the spread of *P. lateralis* (Roth, Harvey, and Kliejunas 1987; Kliejunas and Adams 1981; Roth, Trione, and Ruhmann 1957; Trione 1959). Chlamydospores in decomposed tissue are spread, most often during the wet season, on logging equipment, road graders, commercial and off-road vehicles, and elk, deer, and cattle, from infested to uninfested areas. Water movement along roads, downslope from infected areas, and in streams carries spores a much greater distance. As a result, the disease is most commonly associated with roads, streams, waterways, ditches, and areas where soil water accumulates.

Vegetative spread of the fungus through root grafts is another mode of spread to consider in dense stands of Port-Orford-cedar and yew. Root grafting and infection of fallen green foliage serves as the only means of unassisted uphill spread. Gordon and Roth (1976) found that, in a 50-year-old stand comprised largely of Port-Orford-cedar, a linear relationship existed between root grafting and distance between trees. Frequency of root grafting dropped to nearly zero as distance between trees exceeded six meters.

Spread of *P. lateralis* in the absence of human activity is limited spatially and temporally, by the distance between host trees, by the distance that zoospores can swim, and by the duration of the wet season. Wide natural dispersal of this soilborne obligate parasite cannot occur without the help of man. The topography of the land and patchy occurrence of host trees serve as a natural means to limit spread in protected areas.

Pathogen Detection

Phytophthora lateralis can be successively isolated from infected trees, water, or soil. When a tree is dead several years, recovery by direct isolation is usually unsuccessful, as the margin of infection is difficult to determine, and secondary organisms such as *Pythium* follow closely behind *P. lateralis*, hindering species identification. ELISA (enzyme-linked immunosorbent assay) can be used as a quick test to detect *P. lateralis* in infected tissue of any age, including long dead Port-

Orford-cedar trees, and possibly in Pacific yew as well. *Phytophthora lateralis* can be detected in soils and streams by baiting the fungus with cedar foliage or seedlings (Hamm and Hansen, Report on isolation and identification of *Phytophthora* from conifers in the Pacific Northwest. Department of Botany and Plant Pathology, Oregon State University, 1984).

The simplest means of isolation is from recently dead host tissue. Tucker and Milbrath (1942) sampled tissue taken from the active margin of infection on the inner bark of Port-Orford-cedar and plated 1-2 mm^2 sections onto potato dextrose agar. Currently, root tissue is surface sterilized in a 1:4 bleach to water solution, rinsed in distilled water, and plated onto selective media.

To determine cause of death for trees dead several years, an ELISA test has proved to be beneficial. A 96-well double-antibody sandwich immunoassay kit, manufactured by Neogen, Corp. (Lansing, MI), performs well in a laboratory setting. ELISA works on the principle of antibody-antigen binding. Each individual well is coated with purified antibodies that are specific to proteins of the target plant pathogen. The test material is added to each well, and if present, antigen molecules will bind to the antibody molecules on the well. Enzyme-conjugated antibody is then added to the wells, and binds to the antigen, forming a "sandwich". After incubation and removal of excess conjugate, a substrate is added that changes color in the presence of the enzyme. The color development is proportional to the amount of pathogen proteins in the original test material. Hansen (Final Report on Port-Orford-cedar Evaluation, 1992) tested Neogen's Phytophthora Kit on long dead Port-Orford-cedar. Tissue was taken from both the root collar and from approximately 6 feet up from the tree bole, where *P. lateralis* should not occur. The immunoassay results coincided with predicted results based on visual observations of each tree. Some species of *Pythium*, including *P. ultimatum*, *P.*

aphanidermatum, and *P. dissotocum*, can show low levels of reactivity in the assay (Williams, Regional Manager, Neogen, Corp., pers. comm. 1994)

Phytophthora lateralis is isolated from soil by floating cedar branchlets over soil organic matter and then plating the branchlets onto selective media. Ostrofsky, et al. (1977) assayed soil from infested sandy, silty, and clay loam soils, as well as artificially infected greenhouse soil. Port-Orford-cedar roots, branchlets, and seedlings were used as baits, and, in petri dishes, were floated over organic matter sieved from the infested soil. The branchlets and seedlings yielded the best results, possibly due to the physiologically young tissue, and *P. lateralis* was recovered from all soil types.

In a slightly improved baiting method, Hamm and Hansen (1984) used hymexazol to inhibit *Pythium* growth. Five grams of organic matter sieved from infected soil were placed in the bottom of a styrofoam cup containing a second styrofoam cup with cheesecloth replacing its base. Five Port-Orford-cedar branchlets, 1-2 cm long, were floated over the organic matter in distilled water containing 25 g/L hymexazol to inhibit *Pythium* infection on the branchlets. After 6 days, the branchlets were plated onto selective media and incubated in the dark for 3-4 days. *Pythium* infections on baits and growth of non-Oomycete fungi on selective media were greatly reduced. *Phytophthora lateralis* can thus be isolated at any time of the year.

Port-Orford-cedar branches or seedlings whose roots emerge from plastic growth tubes have also been used as successful baits for *P. lateralis* in natural water sources. Based on the principle of chemotaxis, the zoospores will be attracted to and invade the substrate. This procedure is much more time consuming but, according to Hansen (Oregon State University, pers. comm. 1993), has proven to be highly specific. Seedlings in the tubes are immersed for approximately 7-10 days and then greenhouse incubated for 2-6 weeks. Symptoms are monitored and direct isolations from the roots confirm or deny presence of *P. lateralis*.

Disease Management

In 1986, the North Coast Environmental Center and the Oregon Natural Resources Council employed the Western Natural Resources Law Clinic to urge the Forest Service to implement a region-wide management program to prevent spread of the fungus, to continue research, and to consider cumulative impacts throughout Regions 5 and 6. They suggested that these goals be met through the formation of an Inter-Regional Port-Orford-cedar Committee whose primary goal would be "to identify protectable stands of Port-Orford-cedar, to which access must be indefinitely prohibited" (Axline and Suagee, Correspondence to Regional Foresters in Regions 5 and 6 from Western Natural Resources Law Clinic, 1986).

Regional foresters developed the Inter-Regional Coordinating Group comprised of representatives from the Six Rivers, Siskiyou, Rogue River, and Umpqua National Forests, Forest Pest Management, Bureau of Land Management, and research. Four areas of concern addressed by the Group were: a) inventorying and monitoring; b) research; c) public involvement and education; and d) management policy (Barker and Torrence 1988).

By 1993, the distribution of Port-Orford-cedar was mapped and incorporated into a Geographic Information System database, including areas of *P. lateralis* infection. Monitoring disease spread during timber and other project activities in infested areas occurs within ranger districts of the Siskiyou and Six Rivers National Forests. Research has focused on detection and quantification of fungal propagules in soil and water, and host resistance. A screening program at Oregon State

University is underway to test resistance of selected parent trees chosen from the field along with a number of their half-sib progeny. Newsletters, road signs, pamphlets, slide presentations, and interagency meetings were all underway by 1991 for technology transfer and to initiate public involvement. Management policies were initiated that included developing strategies for timber sale contracts and sale preps, road building and management, reforestation and timber stand improvement, and development of a site-by-site risk rating scale.

Today, prevention of fungal spread is the primary means of controlling *P. lateralis* root rot. Regions 5 and 6 of the US Forest Service have established management practices that include check stations for washing trucks and logging equipment free of soil; building road berms, closing contaminated public use areas, limiting logging practices to the dry season and closing certain roads during the wet season. Effectiveness of these control strategies has yet to be determined.

THESIS OBJECTIVES

We believe that Pacific yew is less susceptible to *P. lateralis* than is Port-Orford-cedar, and that areas where yew mortality is high can be characterized by the presence of slow moving water and adjacent, *P. lateralis*-killed trees. The following objectives will be addressed in this thesis:

1. Compare susceptibility of Pacific yew to Port-Orford-cedar after root and stem inoculations with *P. lateralis*.

2. Compare differences in *P. lateralis* zoospore attraction to Pacific yew and Port-Orford-cedar rootlets.

3. Measure and compare current mortality of Pacific yew and Port-Orford-cedar in *P. lateralis*-infected drainages.

4. Compare local environments of Pacific yew trees killed by *P. lateralis* and live yew trees in the same vicinity.

Chapter 2. The Relative Susceptibility of Pacific Yew and Port-Orford-Cedar to *Phytophthora lateralis*

INTRODUCTION

Pacific yew (*Taxus brevifolia* Nutt.) is a conifer native to the Pacific Northwest and regions of the Rocky Mountains. It is a little-studied tree species that provides food and cover for wildlife, shades stream bottoms, and contributes to stream channel stabilization through its fibrous root system. Wood of the Pacific yew was valued by Native Americans for making tools and hunting implements, and is still used today (Hartzell 1991). Pacific yew gained short-lived fame after a report that a chemical extracted from its bark, taxol, had anti-tumor activity (Wani, et al. 1971). For a period of several years, this tree was heavily harvested on state and federal lands for taxol extraction. The exploitation of this species eventually led to the need for a Federal Environmental Impact Statement, completed in September of 1993. Taxol can currently be totally synthesized (Nicolaou, et al. 1994), reducing the demands on Pacific yew.

Pacific yew is now no longer in danger of over-harvest, but the recent report of a new pathogen focuses attention once again on this species. *Phytophthora lateralis* Tucker and Milbrath, once thought to be a host specific pathogen on Port-Orford-cedar (POC) (*Chamaecyparis lawsoniana* (A. Murr.) Parl.), was reported on Pacific yew in 1991 (DeNitto and Kliejunas). The pathogen is believed to be introduced to the Pacific Northwest based on its aggressive pathogenicity on POC and the fact that little resistance has been seen in the field. The population of POC has been severely affected in its native range, and the ornamental business has been nearly eliminated. Monetary losses range in the millions of dollars, and ecosystem function in riparian areas may be altered due to loss of the cedar overstory. The pathogenicity of *P. lateralis* on POC has been well documented (Trione 1959; Roth, Trione, and Ruhmann 1957; Tucker and Milbrath 1942). Little is known, however, about the infection biology of the fungus on Pacific yew, or the susceptibility of this new host to the pathogen.

Phytophthora lateralis was first noted on POC nursery seedlings in 1923 (Zobel, Roth, and Hawk 1985) near Seattle, Washington. However, it wasn't until 1942 that the causative agent was named and described (Tucker and Milbrath 1942). By 1952, out-plantings of infected cedar ornamentals had spread the fungus from nurseries to the northern portion of the native range of POC, which extends from southwest Oregon into northwest California (Roth, Trione, and Ruhmann 1957). Today, the fungus has been spread almost entirely throughout the range of the cedar, concentrated along roads and waterways and often associated with forest management activities (Zobel, Roth, and Hawk 1985). An Interregional Port-Orford-cedar coordinating group was established in 1986 for the management and restoration of cedar habitat, and for research focusing on resistance to the pathogen.

No management activities have been outlined for Pacific yew in *P. lateralis*-infested areas due to the minor amount of reported mortality in the field, as compared to cedar mortality. In 1991, *P. lateralis* was isolated from 17 of 44 dead yew trees in a U.S. Forest Service field survey covering infested drainages in much of the native range of POC (DeNitto, USDA Forest Service, pers. comm.). The overall extent of mortality of Pacific yew throughout the range of POC has not been determined.

This study is the first to examine the host-pathogen system of Pacific yew and *P. lateralis*. The primary objective was to compare the relative susceptibility of Pacific yew and POC to infection by *P. lateralis*, with a working hypothesis that Pacific yew is less susceptible than POC. In order to provide better information for forest managers in areas where POC and Pacific yew occur together, the following experiments were performed using the two host species: 1) root inoculations on seedlings and rooted cuttings, 2) branch and stem inoculations, and 3) zoospore attraction to rootlets. Comparisons were made between root inoculation methods to provide a recommendation for a satisfactory method of inoculating Pacific yew in future studies. In addition, a field survey of *P. lateralis*-infested drainages in northwest California and southwest Oregon was conducted to examine differences between and Pacific yew mortality within drainages, and to determine the extent of mortality within populations of Pacific yew.

METHODS

Root Inoculations

Fungal Cultures

Two isolates of *P. lateralis* were used for the inoculation studies. Isolate 366 was collected in 1986 from a POC tree on the Gasquet Ranger District of Six Rivers National Forest in northern California, (Kliejunas, USDA Forest Service, pers. comm.) and recovered from liquid nitrogen storage at Oregon State University (OSU) in 1992. Isolate 91-9-9-2 was collected in 1990 from a Pacific yew tree in northwest California (DeNitto, USDA Forest Service, pers. comm.). Isolates were maintained on corn meal agar at 25 C in the dark, and subcultured biweekly.

Seedlings and Cuttings

Plant material was chosen on the basis of availability. The Weyerhaeuser Nursery in Centralia, Washington, provided 200 Pacific yew seedlings and the Stone Nursery in Medford, Oregon, provided 100 yew seedlings and 100 yew cuttings. The Weyerhaeuser seedlings originated from parent trees outside the range of POC, were approximately 4-yr-old, averaged 65 cm in height, 1.3 cm in diameter at the root collar, and 32 cm in total root length. The Stone nursery seedlings were 2-yr-old, averaged 45 cm in height, 0.4 cm in diameter at the root collar, and 21.1 cm in total root length. The seeds had been collected from trees representing various geographic locations across the southern portion of Pacific yew's native range. The Stone nursery cuttings were 2-yr-old, averaged 35 cm in height, 0.5 cm in diameter at the root collar, and 20.2 cm in total root length. Material for the cuttings originated from two healthy Pacific yew trees growing in a *P. lateralis*-infested tributary of the Smith River, located on the Gasquet Ranger District of Six Rivers National Forest, California. All of the POC seedlings were provided by Dr. Everett Hansen, OSU, and averaged 4.5 yr in age, 95 cm in height, 1.5 cm in diameter at the root collar, and 26.6 cm in total root length.

All plant material remained in the greenhouse, which was maintained at temperatures of 24 C during the day and 15 C at night, without supplemental light sources.

Experimental Design

Each POC and Pacific yew seedling or cutting was randomly assigned one of four inoculation treatments: 1) root dip, 2) chlamydospore, 3) planting alongside a diseased POC (InfPOC), and 4) planting in soil amended with infected POC rootlets

(InfSoil). In addition, control inoculations were applied to a random selection of seedlings and cuttings from each species group for each inoculation treatment to determine if factors other than *P. lateralis* could be causing damage. Inoculation treatments were performed independently of each other due to the amount of labor and time involved in preparing inoculum and reading results. Furthermore, within each treatment, inoculations were performed on two separate dates due to Pacific yew seedling availability. The Weyerhaeuser seedlings were provided first, and after several months, over 70 were killed or damaged through larval root feeding of the black vine weevil (*Otiorhynchus sulcatus*). The remaining healthy seedlings, along with POC seedlings, were inoculated via each treatment. The lost seedlings were replaced by plants from Stone nursery five months later, and were inoculated, also along with POC seedlings, via each treatment. The numbers of seedlings and cuttings included within each inoculation treatment are shown in Table 1.

Table 1. Sample size of inoculated and control Pacific yew and Port-Orford-cedar seedlings and cuttings, by inoculation treatment.

	Inoculation Treatment			
Plant Group[1]	**Root Dip**	**Chlamydospore**	**InfPOC**	**InfSoil**
Weyerhaeuser Pacific yew				
seedlings inoculated	10	10	14	13
seedlings control	3	3	3	3
Port-Orford-cedar				
seedlings inoculated	8	8	6	6
seedlings control	2	2	2	2
Stone Pacific yew				
seedlings inoculated	15	15	10	11
seedlings control	3	3	3	3
cuttings inoculated	20	20	20	20
cuttings control	6	6	6	6
Port-Orford-cedar				
seedlings inoculated	8	10	6	6
seedlings control	4	4	4	2

[1]The Weyerhaeuser Pacific yew and first group of POC seedlings were inoculated 5 months before the Stone seedlings and cuttings and second group of POC seedlings.

Root Dip Inoculation

Inoculum was prepared in equal amounts from isolates 366 and 91-9-9-2 by growing four *P. lateralis* agar discs cut from the leading edge of actively growing mycelium in a 100 x 15 mm petri plate containing split pea broth amended with 20 ppm β-sitosterol (Englander and Roth 1980) for 7 days in the dark at 17 C. Mycelial colonies within each plate were then rinsed with distilled water, flooded with 20 ml of filter sterilized water originating from Oak Creek in Corvallis, Oregon, and incubated at 17 C in the dark for 24 h. Zoospore emergence typically began 17-20 h after addition of the Oak Creek water. Initial inoculum concentrations were determined through duplicate counts of every tenth plate using a hemacytometer, and averaged 2.3×10^4 spores/ml for the Weyerhaeuser/POC inoculation, and 1.9×10^4 spores/ml for the Stone/POC inoculation.

The root dip inoculation technique consisted of removing seedlings and cuttings from their potting soil, cutting the terminal root ends to a length of 13-14 cm, and suspending the root tips of three seedlings of the same species in a 1 liter plastic container holding 40 ml of zoospore suspension (20 ml from each isolate) plus 100 ml distilled water. The control inoculation consisted of cutting the root ends and placing the seedlings and cuttings in plastic containers holding 140 ml distilled water. Aluminum foil was wrapped around the containers to keep the light out, and after 24 h the seedlings and cuttings were repotted and placed on the greenhouse bench for 15 wk where they were watered every 3-4 days.

Chlamydospore Inoculation

Inoculum was prepared in equal amounts from isolates 366 and 91-9-9-2 by growing four *P. lateralis* agar discs cut from the leading edge of actively growing mycelium in each of 20, 100 x 15 mm petri plates containing 20 ml split pea broth amended with 20 ppm β-sitosterol for 15-20 days in the dark at 25 C. All mycelial colonies were then rinsed with distilled water, combined in a metal container, macerated with a household blender for 10-15 minutes, and filtered twice, first through a 1 mm^2 metal sieve, then through a one-ply cheesecloth. Chlamydospore concentrations were determined using a hemacytometer, and averaged 1.2×10^3 spores/ml for the Weyerhaeuser/POC inoculation and 1.1×10^3 spores/ml for the Stone/POC inoculation.

The chlamydospore inoculation technique consisted of pouring 1 ml of the chlamydospore inoculum directly into each of three 1 x 1 x 9-cm-deep holes around the stem of the potted seedling or cutting. Distilled water replaced the chlamydospore inoculum for the control inoculated seedlings and cuttings. After inoculation, each pot was water saturated in a 19 L (5 gal) bucket for 24 hr to induce sporangia and zoospore release. Seedlings and cuttings were then placed on the greenhouse bench for 15 wk and watered every 3-4 days.

InfPOC Inoculation

Port-Orford-cedar seedlings were inoculated using the root dip method, and when the distinct *P. lateralis* infection margin appeared at the root collar, the infected seedlings were used as inoculum source. Each healthy seedling or cutting was planted into a one gallon pot adjacent to an infected cedar seedling, while control inoculated seedlings and cuttings were planted adjacent to a healthy POC seedling. Each pot was placed in a 19 L bucket containing water for 24 hr, once a week for 12 wk to induce sporangia and zoospore release from the infected cedar tissue. The seedlings and cuttings then remained on the greenhouse bench an additional 3 wk after the water saturation period, where they were watered weekly.

InfSoil Inoculation

Port-Orford-cedar seedlings were inoculated with the root dip method, and when the distinctive *P. lateralis* infection margin appeared at the root collar, chopped roots from the infected seedlings were used to inoculate soil into which healthy seedlings and cuttings were planted. The infected roots were chopped into 3 cm long pieces and mixed with soil, 100 g of root inoculum per 4 L (1 gal) pot. Cedar and yew seedlings and cuttings were then planted, one per pot. Check inoculations consisted of planting seedlings or cuttings into soil amended with 100 g of healthy cedar rootlets. All pots were then individually water saturated in 19 L buckets for 24 hr once a week for 12 wk to induce sporangia and zoospore release from the infected root tissue. The seedlings and cuttings then remained on the greenhouse bench for an additional 3 wk with weekly watering.

Data Collection and Recovery of *P. lateralis*

Fifteen wk after the inoculation treatments, the root mass of each seedling and cutting was examined. Percent lesion length, percent necrotic root tips, a rating of disease on the root ball based on percent visible dead roots, and plant survival were measured. Lesion length was determined for each seedling and cutting by dividing the root mass into one to three main sections, exposing the phloem to locate a margin between healthy and diseased tissue, and measuring the longest lesion extension within each main section. The average lesion extension was then determined, if necessary, and converted to a percent based on the total length of the root mass, measured from the root collar to the root tips. Percent necrotic root tips was determined by counting the number of necrotic root tips and dividing by the total number of root tips. Root masses were assigned a rating of percent visible necrotic root tissue from 0 to 4 (0=0-10%, 1=11-25%, 2=26-50%, 3=51-75%, and 4=76-100%). Survival was recorded as positive if the lesion had not reached and girdled the root collar.

Fungal isolations were performed using the double cup baiting method (Hamm and Hansen 1984) on roots of all Pacific yew seedlings and cuttings, and on a random selection of POC seedlings and control inoculated seedlings and cuttings after recording root measurements. Five root pieces from each seedling were excised from areas where necrotic and healthy phloem tissue converged. Root pieces were then placed in one styrofoam cup per seedling or cutting, filled with 100 ml distilled water amended with 25 ppm hymexazol, on which four 1-cm-sections of fresh POC foliage floated as a bait. After 7 days, the foliage was plated onto a selective medium containing corn meal agar, 10 ppm pimaricin, 250 ppm ampicillin, and 10 ppm rifampicin. Plates were examined under a dissecting microscope for growth of *P. lateralis* 3-4 days after incubation at 25 C in the dark.

Data Analysis

The two groups of POC seedlings inoculated within each treatment (see Table 1) behaved similarly, as evidenced by the non-significant *P*-values of Student's t-statistics (Table 2). Thus for the purposes of the analyses, results of all cedar seedlings within each inoculation treatment were combined, and comparisons made among the four groups: the Weyerhaeuser yew seedlings, Stone yew seedlings, Stone yew cuttings, and POC seedlings. Two-way ANOVA tables were constructed to evaluate differences in percent lesion length and percent necrotic root tips (logit transformed to achieve equal variance), and percent survival, within and between inoculation treatments. Individual means were compared using Fisher's protected least significant difference test ($P = 0.05$). Root rot percent ratings were at times all or mostly "0" for the yews, or all or mostly "4" for the cedars, causing difficulties in data analysis. As a result, the rating of each seedling was converted to the mean percent root mass necrosis for that rating (0=5%, 1=18%, 2=38%, 3=63%, and 4=88%). The final value was transformed via an arcsin square root function to achieve equal variance, and comparisons made between species groups using ANOVA using Statistical Analysis Systems version 6.08 (SAS Institute, Cary, NC). In addition, results for POC and Pacific yew groups were compared between inoculation treatments to determine which method resulted in the greatest amount of damage for each group.

Table 2. *P*-values as determined by Student's t-test, obtained from comparison of four response variables among the first and second groups of inoculated Port-Orford cedar seedlings for each inoculation treatment.

	Inoculation Treatment			
Response Variable	**Root Dip**	**Chlamydospore**	**InfPOC[2]**	**InfSoil[3]**
%Lesion Length	0.334	0.918	0.754	0.864
%Necrotic Root Tips	0.186	0.443	0.513	0.192
%Root Mass Necrosis	------[1]	0.631	0.268	0.581
%Survival	------[1]	0.693	0.599	1.000

[1]Values were identical for the two groups.
[2]Planted alongside a *P. lateralis*-infected POC seedling
[3]Planted in soil amended with *P. lateralis*-infected rootlets

Results of the controls were not included in the ANOVA due to the high number of zeros recorded for several variables, but, for comparison, means of control treatments were tabulated with inoculated treatments.

Branch and Stem Inoculations

Branch and Stem Descriptions

Fifteen healthy POC and thirty healthy Weyerhaeuser Pacific yew seedlings were chosen for stem inoculations, all originating from the same group of seedlings used in the root inoculations. Additionally, branches were excised from trees in two geographic locations, MD and EC. The MD source trees included six Pacific yew trees in MacDonald Forest near Corvallis, OR, and one POC tree on the OSU campus, while the EC source trees included seven Pacific yew trees and two POC trees growing stream-side in the infested Elder Creek drainage on the Illinois Valley Ranger District in Siskiyou National Forest. Trees were chosen on the basis of a healthy appearance, accessibility, and presence of useful branches. Five of the youngest, one meter-long branches without forks were excised from each selected tree and bagged and labeled by tree and transported to the OSU campus within 2 to 8 hr. In the greenhouse, all branches were grouped by tree and the cut ends immersed in 19 L buckets of water where they were inoculated within two days.

Experimental Design

Inoculation of seedling stems, and MD and EC branches was arranged as a two by three factorial of two species treatments and three sources of plant material. Due to the difficulty of collecting all branches at once, inoculations were performed on three separate dates, with each date representing one source of plant material. Control inoculations were performed to determine if factors other than *P. lateralis* were involved in lesion formation.

Inoculation

Inoculum was prepared by growing equal amounts of isolates 366 and 91-9-9-2 in each petri dish containing pea broth for 7 days in the dark at 17 C. Stem inoculations were performed on intact seedlings at least 5-10 cm above the soil line. Branch inoculations occurred at mid-stem, where the diameter was between 4 mm and 1 cm, where few lateral branches grew, and where the advancing lesion would not extend into the portion of the branch resting in water. The seedling stem or branch bark was cut longitudinally to a length of approximately 1-1.5 cm, and to the depth of the xylem. A 1 mm^3 section of actively growing mycelium was inserted into the wound and covered with petroleum jelly. As controls, one branch from each tree and four seedling stems from each host were cut and sealed with petroleum jelly, but received no inoculum.

Data Collection and Analysis

Six weeks following inoculations, the inner bark was mechanically exposed and the extent of the visible lesion on the branch or stem was recorded. Comparisons of log-transformed lesion lengths were made among and within the Pacific yew and POC branches and stems by constructing a two-way ANOVA table with contrasts to compare individual and grouped means using Statistical Analysis Systems version 6.08 (SAS Institute, Cary, NC).

Zoospore Attraction to Rootlets

Experimental Design

Inoculum for this experiment was prepared identically to the root dip method, with equal amounts of isolates 366 and 91-9-9-2. Five 1-cm-long, feeder rootlets were cut from both a single cedar and single yew seedling, and two feeder rootlets were cut from one Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedling as a control. Plates were assembled by floating a rootlet on 10 ml of the zoospore inoculum (5 ml from each isolate) in a 60 x 15 mm petri plate. Plates were assembled five minutes apart to allow time for rootlet examination, and the process was repeated three times. Zoospore concentrations for experimental replications 1-4, determined with a hemacytometer, averaged 1.6 x 10^5 spores/ml, 1.7 x 10^5 spores/ml, 1.8 x 10^5 spores/ml, and 1.7 x 10^5 spores/ml.

Data Collection and Analysis

A compound microscope was used to examine the number of encysted zoospores on the rootlet sections at 15 min, and 1 and 4 hr. Zoospores tended to encyst in layers around the root circumference, which made determining exact numbers impossible, so a rating system of spores per root section from 0-4, where 0=0-10 spores, 1=11-100 spores, 2=101-500 spores, 3=501-1000 spores, and 4=>1000 spores, was established. A rating was given to each of the following four sections of each root sample: the root cap, the zone of elongation, the region of maturation, and on the surface of the cut end. The rootlet segments were not of equal lengths (Figure 1).

Data were analyzed via a generalized linear model of the poisson distribution of rating using Statistical Analysis Systems version 6.08 (SAS Institute, Cary, NC). Data for the Douglas-fir control rootlets were not included in the analysis because the rating was often "0". Comparisons were made within and among species for each location along the rootlet.

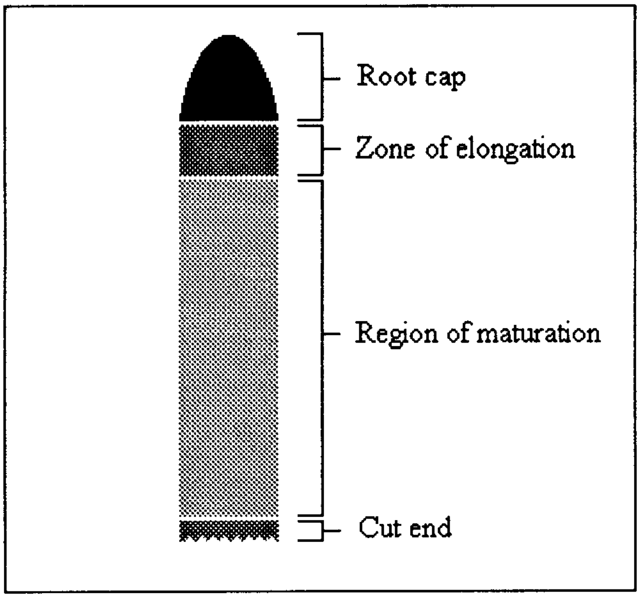


Figure 1. Schematic of rootlet, specifying each division in which zoospore encystment ratings were applied.

Stream Survey

Stream Selection and Descriptions

In the summer of 1993, 0.8 km (0.5 mi.) sections of three streams in southwest Oregon and northwest California were surveyed for live and dead POC and Pacific yew (Table 3). The streams surveyed included the upper reaches of the Middle Fork of the Smith River, and an unnamed tributary of Coon Creek which flows into the South Fork of the Smith River, both located on the Gasquet Ranger District of Six Rivers National Forest, northern California, and Elder Creek, located on the Illinois Valley Ranger District of Siskiyou National Forest, southwest Oregon. The drainages were chosen based on a known history of root rot, and the current presence of unsalvaged, mature POC and Pacific yew.

Table 3. Descriptions of streams and segment locations included in the survey of live and *Phytophthora lateralis*-killed Port-Orford-cedar and Pacific yew trees and seedlings.

Stream	Legal Description	Predominant vegetation	Percent Cover[1]	Stream Width (m)	Gradient[2]
Middle Fork of the Smith River	T17N R5E Sec.6	*Abies*, *Salix* spp, *Gaultheria shallon*, *Vaccinium* spp., grasses, *Rosa* spp.	15	6-12	0-20
Coon Creek	T16N R2E Sec.21	*Pseudotsuga menziesii*, *Arbutus menziesii*, *Salix* spp., *Lithocarpus densiflorus*, *Rosa* spp., *Vaccinium* spp., *Amelanchier* spp., *Arctostaphylos patula*	25	1.5-6	5-30
Elder Creek	T40S R7W Sec.19 and 20	*Pinus lambertiana*, *Calocedrus decurrens*, *Vaccinium* spp., *Castanopsis*, *A. menziesii*, grasses	70	1.5-4.5	5-15

[1]Percent cover of canopy averaged over the length of the survey segment, estimated visually.
[2]Gradient is the slope of the stream channel, measured with a clinometer, and recorded as a percent.

The Middle Fork of the Smith River was the largest stream sampled, with a width up to 12 m. The segment sampled flows through a floodplain where cedar mortality has occurred up to 30 m from the river's edge. The terrain is nearly level, with a stream gradient ranging from 0-20 percent, until the approximate end of the segment, where the river enters a steep canyon. The overstory is dominated by Douglas-fir and POC, with the occurrence of white fir (*Abies concolor*), willow (*Salix* spp.), salal (*Gaultheria shallon*), and huckleberry (*Vaccinium* spp.) Past management history includes clearcutting and road-building dating back to the 1960's. *Phytophthora lateralis* was discovered there in 1988, but was most likely introduced in 1986 after a pre-commercial thinning operation (Wells, USDA Forest Service, pers. comm.).

The segment at Coon Creek begins at an intermittent drainage and continues up and down the intersection with another small but permanent drainage where water flows underground for some portion during the dry season. The width of Coon Creek varies from 1.5 to 6 m, and the gradient of the drainage bottom ranges from 5-30 percent. The overstory is dominated by large POC and Douglas-fir, with the occurrence of willow (*Salix* spp.), madrone (*Arbutus menziesii*), manzanita (*Arctostaphylos patula*), huckleberry (*Vaccinium* spp.), and serviceberry (*Amelanchier* spp.). *Phytophthora lateralis* was known to be in the area by 1984 from unknown introduction, however management activities in the past have included mining and wood cutting (Wells, USDA Forest Service, pers. comm.).

Elder Creek is similar in size to Coon Creek, spanning a width of 1.5 to 4.5 m. The stream occurs in a small canyon, but the terrain along the stream bottom is nearly level, with a gradient of 5-15 degrees. Water flow is year-round but shallow in the portion of stream surveyed. Canopy cover is greatest at this stream at 70 percent, and the overstory is dominated by POC. Incense cedar (*Calocedrus decurrens*), sugar pine (*Pinus lambertiana*), madrone (*Arbutus menziesii*), and golden chinquapin (*Castanopsis* sp.) also occur. The first roads were built in the area in the mid-1960s, and past management includes uphill clearcutting and selective harvesting. *Phytophthora lateralis* was known to be in the area by the mid-1970s (Pera, USDA Forest Service, pers. comm.).

Data Collection and Analysis

Every cedar and yew tree located within 9 m of either side of the seasonal high water line (determined where stream-bank vegetation begins) of each stream was recorded in a size and condition class (1= <12.4 cm diameter, 2= >12.5 cm diameter, and 0=live, 1=dead due to *P. lateralis*). On recently killed trees, the inner bark was examined at the root collar for *P. lateralis* staining if cause of death was questionable.

A generalized linear model of the binomial distribution of total to dead trees was used to determine if differences among the proportion of dead Pacific yew and POC exist within these drainages using Statistical Analysis Systems version 6.08 (SAS Institute, Cary, NC).

RESULTS

Root Inoculations

Overall mortality for the POC seedlings was almost 58% while seven of 178, or 4% of all Pacific yew seedlings and cuttings were killed (Table 4). The greatest amount of mortality for both species occurred with the root dip method of inoculation (9% for Pacific yew, 100% for POC), while the least amount of mortality for Pacific yew occurred with the chlamydospore inoculation (0%), and the least amount of mortality for POC occurred with the InfSoil inoculation method (33%). Almost all response variables in all inoculation treatments were significantly greater in the POC seedlings than in any of the Pacific yew groups. Root mass necrosis, and percent lesion length and necrotic root tips of the Weyerhaeuser seedlings were consistently greater than those of the smaller Stone seedlings and cuttings. For the most part, the Stone seedlings and cuttings behaved similarly; root mass necrosis was low, and none were killed. The control seedlings showed no root damage similar to the inoculated seedlings aside from necrotic root tips, which were seen on every control group (Table 4).

Root Dip

All POC seedlings were killed by the root dip method of inoculation, while 40% of the Weyerhaeuser yews were killed, and none of the Stone yews were killed (Table 4). The mean of each response variable for the cedar seedlings was significantly greater than the corresponding mean in each of the Pacific yew groups ($P \leq 0.05$). The Stone cuttings and Stone seedlings behaved similarly over all measurements, and of the three yew groups, the Weyerhaeuser seedlings showed the greatest percent root mass necrosis, percent necrotic root tips, and lesion length.

Chlamydospore

No Pacific yew seedling or cutting was killed by this method of inoculation, but almost 56% of all POC seedlings died (Table 4). Every response variable measured was significantly greater on the POC seedlings than on of each of the Pacific yew groups ($P \leq 0.05$). Aside from percent root mass necrosis, results of the Stone seedlings and cuttings were not similar. Rather, the response variables among the three Pacific yew groups are rather diverse, with the greatest damage occurring in the Stone cutting group.

InfPOC

Forty-two percent of all cedar seedlings died with the InfPOC method of inoculation while significantly fewer yew seedlings died ($P \leq 0.05$) (Table 4). The greatest values for all measurements were again recorded in the cedar seedlings ($P \leq 0.05$), and within the yew groups, in the Weyerhaeuser seedlings. The Stone seedlings and cuttings behaved similarly over all measurements in the InfPOC inoculation method, and one measurement, percent necrotic root tips, was similar over all the plant groups.

InfSoil

Far fewer cedar seedlings (33%) died in the InfSoil method of inoculation than in any other treatment, while mortality for Pacific yew (almost 5%) was second highest over all four inoculations, all representing seedlings from Weyerhaeuser nursery (Table 4). Again, the Stone seedlings and Stone cuttings behaved similarly, and all response variables are greatest on the Weyerhaeuser seedlings ($P \leq 0.05$). Results with Weyerhaeuser seedlings and POC seedlings are similar for percent lesion length and survival, but significantly different in percent necrotic root tips and percent root mass necrosis ($P \leq 0.05$).

Table 4. Means of disease responses and standard deviations (in parentheses) for four groups of seedlings and cuttings of Pacific yew and Port-Orford-cedar (POC) at 15 wk after inoculation with *Phytophthora lateralis* by four different methods.

Species Group	%Lesion Length	%Necrotic Root Tips	%Root Mass Necrosis[1]	%Survival
		Root Dip Inoculation		
Weyer. yew seedlings	63.5b[2] (25.1)	66.7b (19.5)	52.0b (29.8)	60.0b (0.2)
controls	0.0 (0.0)	10.6 (20.1)	0.0 (0.0)	100.0 (0.0)
Stone yew seedlings	18.8a (10.3)	33.4a (9.8)	19.0a (13.2)	100.0a (0.0)
controls	0.0 (0.0)	2.0 (3.2)	0.0 (0.0)	100.0 (0.0)
Stone yew cuttings	22.7a (15.5)	37.5a (16.9)	25.6a (15.8)	100.0a (0.0)
controls	0.0 (0.0)	7.8 (6.4)	0.0 (0.0)	100.0 (0.0)
POC seedlings	99.7c (1.2)	94.5c (7.9)	88.0c (0.0)	0.0c (0.0)
controls	0.0 (0.0)	5.9 (2.2)	0.0 (0.0)	100.0 (0.0)
		Chlamydospore Inoculation		
Weyer. yew seedlings	1.7a (1.3)	12.4ab (4.7)	6.3a (4.1)	100.0a (0.0)
controls	0.0 (0.0)	8.8 (4.7)	0.0 (0.0)	100.0 (0.0)
Stone yew seedlings	4.9a (8.1)	8.8a (9.8)	5.0a (0.0)	100.0a (0.0)
controls	0.0 (0.0)	2.7 (4.6)	0.0 (0.0)	100.0 (0.0)
Stone yew cuttings	23.3b (23.5)	26.1b (24.1)	14.1a (16.6)	100.0a (0.0)
controls	0.0 (0.0)	4.7 (4.1)	0.0 (0.0)	100.0 (0.0)
POC seedlings	62.7c (41.8)	59.2c (33.8)	56.0b (31.4)	44.4b (0.5)
controls	0.0 (0.0)	4.1 (2.1)	0.0 (0.0)	100.0 (0.0)
		InfPOC Inoculation		
Weyer. yew seedlings	42.4b (31.3)	33.0ab (28.5)	29.1b (26.7)	92.8a (0.2)
controls	0.0 (0.0)	16.6 (7.9)	0.0 (0.0)	100.0 (0.0)
Stone yew seedlings	13.8a (9.5)	22.6a (18.6)	8.9a (6.2)	100.0a (0.0)
controls	0.0 (0.0)	9.2 (7.9)	0.0 (0.0)	100.0 (0.0)
Stone yew cuttings	24.7ab (17.7)	26.2a (14.2)	9.8a (10.8)	100.0a (0.0)
controls	0.0 (0.0)	5.4 (6.5)	0.0 (0.0)	100.0 (0.0)
POC seedlings	69.8c (27.8)	46.1b (26.4)	47.2c (20.9)	58.3b (0.5)
controls	0.0 (0.0)	11.2 (6.8)	0.0 (0.0)	100.0 (0.0)
		InfSoil Inoculation		
Weyer. yew seedlings	54.6b (23.4)	49.1b (22.9)	31.6b (17.8)	84.6ab (0.3)
controls	0.0 (0.0)	21.5 (9.6)	0.0 (0.0)	100.0 (0.0)
Stone yew seedlings	16.9a (15.1)	27.6a (10.3)	12.7a (10.5)	100.0a (0.0)
controls	0.0 (0.0)	9.8 (8.4)	0.0 (0.0)	100.0 (0.0)
Stone yew cuttings	23.4a (16.6)	31.4a (12.9)	19.4a (10.8)	100.0a (0.0)
controls	0.0 (0.0)	16.2 (11.8)	0.0 (0.0)	100.0 (0.0)
POC seedlings	66.1b (20.5)	61.2c (16.0)	47.2c (23.4)	66.7b (0.4)
controls	0.0 (0.0)	28.2 (5.2)	0.0 (0.0)	100.0 (0.0)

[1]Root mass necrosis is a mean based on a previously assigned root rot rating: 0=0-10% necrotic, mean=5%; 1=11-25% necrotic, mean=18%; 2=26-50% necrotic, mean=38%; 3=51-75% necrotic, mean=63%; and 4=76-100% necrotic, mean=88%.

[2]Values with the same letter within each response and treatment are not significantly different according to the Fisher's protected least significant difference (P =0.05).

Comparisons Between Inoculation Methods

The greatest amount of mortality and root damage of POC and all Pacific yew groups occurred with the root dip inoculation. This method of inoculation also produced the most consistent results. For the POC seedlings, results of the remaining inoculation treatments are consistent throughout, although the InfSoil method resulted in the least amount of damage. The chlamydospore inoculation method was least effective for the Weyerhaeuser and Stone yew seedlings and the InfPOC method was the least effective for the Stone yew cuttings.

Recovery of *P. lateralis*

No differences in recovery of *P. lateralis* were observed among any of the groups of Pacific yew plant material, so results for all were combined. *Phytophthora lateralis* was recovered from almost 94% of all inoculated cedar seedlings and from almost 90% of all inoculated Pacific yew seedlings and cuttings, including those with no aboveground symptoms, and from none of the controls (Table 5). The lowest recovery occurred after the chlamydospore inoculation, and the greatest recovery after the root dip inoculation. Despite the high recovery from Pacific yew, inoculated seedlings and cuttings that did not die remained asymptomatic aboveground.

Table 5. Number of Pacific yew and Port-Orford-cedar (POC) seedlings, by inoculation treatment, from which roots were taken for baiting of *Phytophthora lateralis*, and percentage of seedlings for which recovery was successful.

	Root Dip		Chlamydospore		InfPOC		InfSoil	
Species Group	No.	% Success	No.	% Success	No.	% Success	No.	% Success
Pacific yew[1]	45	100.0	40	72.5	44	93.1	44	93.1
Pacific yew--c[2]	3	0.0	3	0.0	3	0.0	3	0.0
POC	10	100.0	10	83.6	6	100.0	6	91.7
POC--c	2	0.0	2	0.0	2	0.0	2	0.0

[1]All Pacific yew seedlings and cuttings inoculated are included.
[2]c= controls

Branch Inoculations

The lesion length of *P. lateralis* in Pacific yew stem and branch inoculations averaged 65.8 mm and 22.3 mm, respectively, while the POC stem and branch lesion lengths averaged 96.4 mm and 82.2 mm, respectively (Table 6). All contrasts of lesion length among POC and Pacific yew tissues were significant ($P \leq 0.05$) (Table 7). Results of contrasts also reveal that a significant difference ($P \leq 0.05$) in average lesion length occurred among yew stems and branches and within yew branches (Table 7). The contrast of POC stems and branches did not show a difference. Additionally, a significant difference in lesion length was detected within the Pacific yew branches when the results of the two locations--MD and EC--were compared ($P \leq 0.05$), whereas no significant difference was seen in the same comparison of results within POC branches.

Table 6. Mean lesion length (mm) and standard deviation (in parentheses) of Pacific yew and Port-Orford-cedar (POC) seedling stems and mature tree branches 6 wk after inoculation with *Phytophthora lateralis*.

	Stem			Branch								
				MD[2]			EC[3]			All		
Species	N	Mean	(SD)	N	Mean	(SD)	N	Mean	(SD)	N	Mean	(SD)
Yew	30	65.8	(33.4)	30	29.9	(6.7)	35	15.8	(5.0)	65	22.3	(9.5)
Yew-c[1]	4	10.0	(2.2)	6	9.7	(1.9)	7	9.6	(1.0)	13	9.6	(1.4)
POC	15	96.4	(18.8)	5	86.2	(29.3)	10	80.2	(22.9)	15	82.2	(24.3)
POC-c[1]	4	10.7	(1.2)	2	12.0	(0.7)	2	11.0	(1.4)	3	12.0	(2.0)

[1]control branches and stems

[2]MD=branches taken from Pacific yew trees in MacDonald-Dunn Experimental Forest, and from POC on the OSU campus

[3]EC=branches taken from Pacific yew and POC trees in Elder Creek, Siskiyou National Forest

Table 7. ANOVA table and contrasts comparing the logarithm of lesion length caused by *P. lateralis* on Pacific yew and Port-Orford-cedar (POC) stems and branches.

Source of variation	DF	Sum of squares	Mean square	F value	Pr > F
Model	11	92.613	8.423	74.090	0.001
Error	137	15.572	0.110		
Corrected total	148	108.184			

Source of variation	DF	Type I SS	Mean square	F value	Pr > F
Species	3	62.900	20.972	184.521	0.001
Origin	2	20.274	10.135	89.183	0.001
Species*Origin	6	9.441	1.574	13.843	0.001

Contrast	DF	Contrast SS	Mean square	F value	Pr > F
POC v. yew-MD[1]	1	2.621	2.621	23.073	0.001
POC v. yew-EC	1	6.378	6.378	56.036	0.001
POC v. yew-all branch	1	10.313	10.313	90.776	0.001
POC v. yew-stem	1	15.032	15.032	132.285	0.001
MD v. EC-yew	1	7.163	7.163	63.051	0.001
MD v. EC-POC	1	0.014	0.014	0.128	0.728
stem v. all branch-yew	1	21.010	21.010	185.673	0.001
stem v. all branch-POC	1	0.205	0.205	1.811	0.181

[1]MD branches originated from MacDonald-Dunn Forest and the OSU campus; EC branches originated from Elder Creek, Siskiyou National Forest

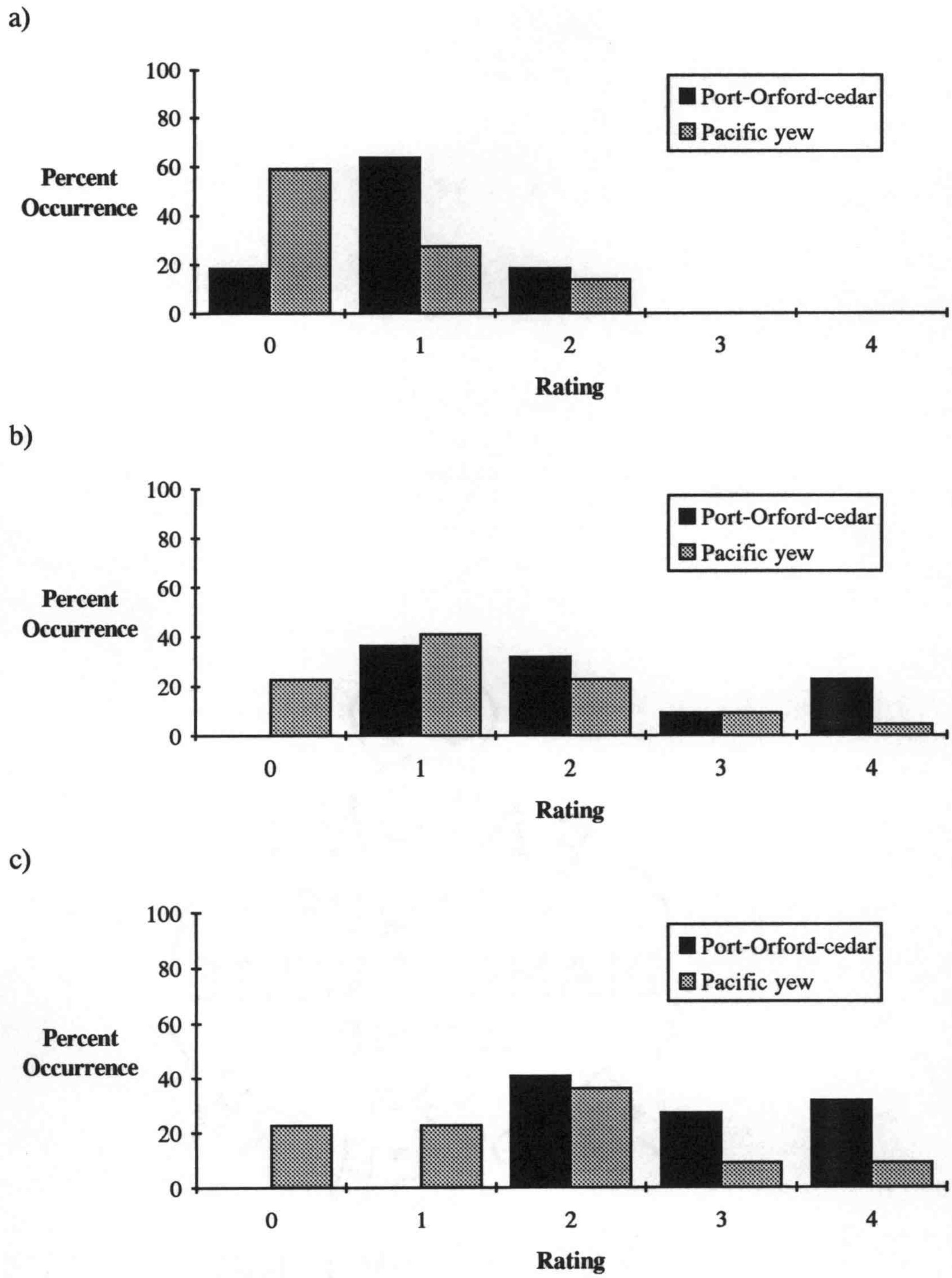
Zoospore Attraction to Rootlets

The total number of zoospores that encysted on POC rootlets was consistently greater than the number of zoospores encysting on the Pacific yew rootlets over every time period (Figure 2a-c), although initially, on some specific regions of the rootlets such as the cut end (Figure 3a), similar numbers of zoospores encysted on both host species. By the four hour time period, the number of zoospores that had encysted on POC were significantly greater than on Pacific yew over three of the four rootlet segments.

Locations along the cedar and yew rootlets where zoospores encysted varied between the two species. On the cedar rootlets, zoospores more commonly encysted on the zone of elongation (ZOE) and in specific areas along the region of maturation (ROM) in layers of up to 10 deep. Zoospores were constantly swarming around the rootlet, and those that did not encyst on the root encysted on the bottom of the dish just below where the rootlet floated. On the yew rootlets, however, zoospores more commonly encysted in clumps on root hairs (included in the ROM count), but did not seem to aggregate more specifically in one area than in another. Zoospores did not aggregate or swarm specifically around the Douglas-fir control rootlets, and those that did encyst on the rootlet itself may have done so by chance, for there was no pattern or preference of zoospore encystment anywhere on the root or on the bottom of the petri dish near the root.

After the first fifteen minutes of zoospore exposure, the only areas on the yew rootlets where significant numbers of zoospores encysted occurred along the ROM and at the ZOE (Figures 3a-6a). No significant difference was seen between the Pacific yew and POC counts on any root segment except the root cap ($P \leq 0.05$, Table 8). For the count of total zoospores on the rootlets, however, a far greater number of zoospores ($P \leq 0.05$) had encysted on the POC rootlets than on the yew rootlets (Table 8). Within the Pacific yew counts, the numbers of spores encysting along the ROM were significantly greater than on any other portion of the rootlet ($P \leq 0.05$); whereas, no differences were seen within the POC zoospore counts when comparing across rootlet segments (Table 9). After one hour, the difference was more clearly seen between the numbers of zoospores on the Pacific yew and POC rootlets (Figures 3b-6b, and Table 8). A far greater number of zoospores encysted on the cedar ZOE, root cap, and cut end than on the same areas on Pacific yew (P

$\leq$0.05). On the ROM of the two species, only slightly more zoospores encysted on the cedar rootlets. Results of the one hour readings for zoospore counts within species are similar to results of the fifteen minute reading (Table 9). Results of the final reading show the clear distinction between the numbers of zoospores encysting on Pacific yew as compared to POC (Figures 3c-6c, and Table 8). Significant differences were seen on all rootlet segments aside from the ROM ($P \leq 0.05$). Within both species, the least amount of zoospores encysted along the cut end, and on the POC rootlets, the greatest concentration of encysted zoospores occurred at the ZOE, while on Pacific yew, the greatest concentration of encysted zoospores occurred along the ROM (Table 9).



Figures 2a-c. Frequency with which Port-Orford-cedar and Pacific yew rootlets received each rating from 0-4 (0=0-10 zoospores, 1=11-100 zoospores, 2=101-500 zoospores, 3=501-1000 zoospores, 4=>1000 zoospores) over the **entire rootlet**, at a) 15 minutes, b) 60 minutes, and c) 240 minutes after exposure to zoospores of *P. lateralis*.

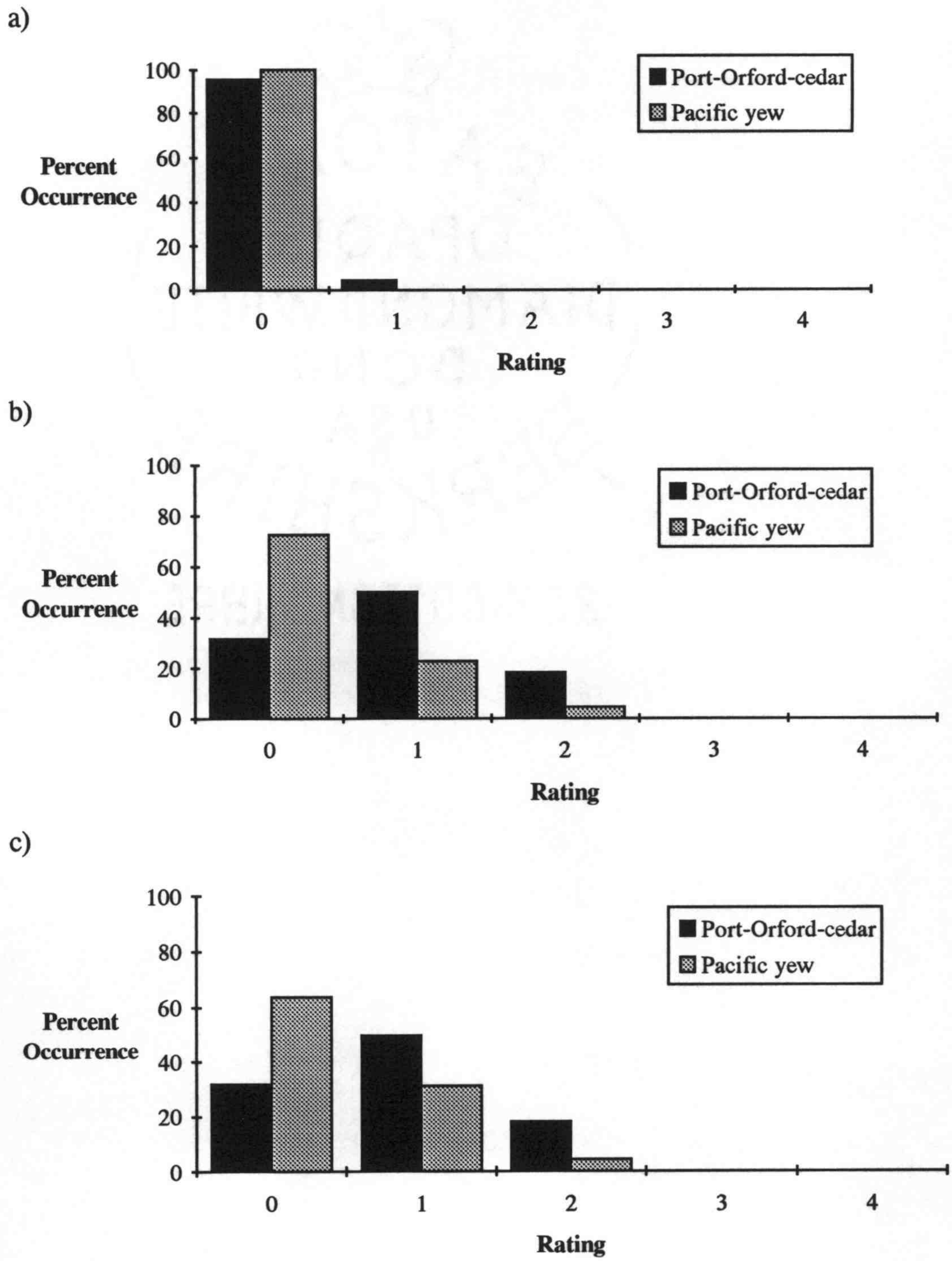


Figure 3a-c. Frequency with which Port-Orford-cedar and Pacific yew rootlets received each rating from 0-4 (0=0-10 zoospores, 1=11-100 zoospores, 2=101-500 zoospores, 3=501-1000 zoospores, 4=>1000 zoospores) at the **cut end**, at a) 15 minutes, b) 60 minutes, and c) 240 minutes after exposure to zoospores of *P. lateralis*.

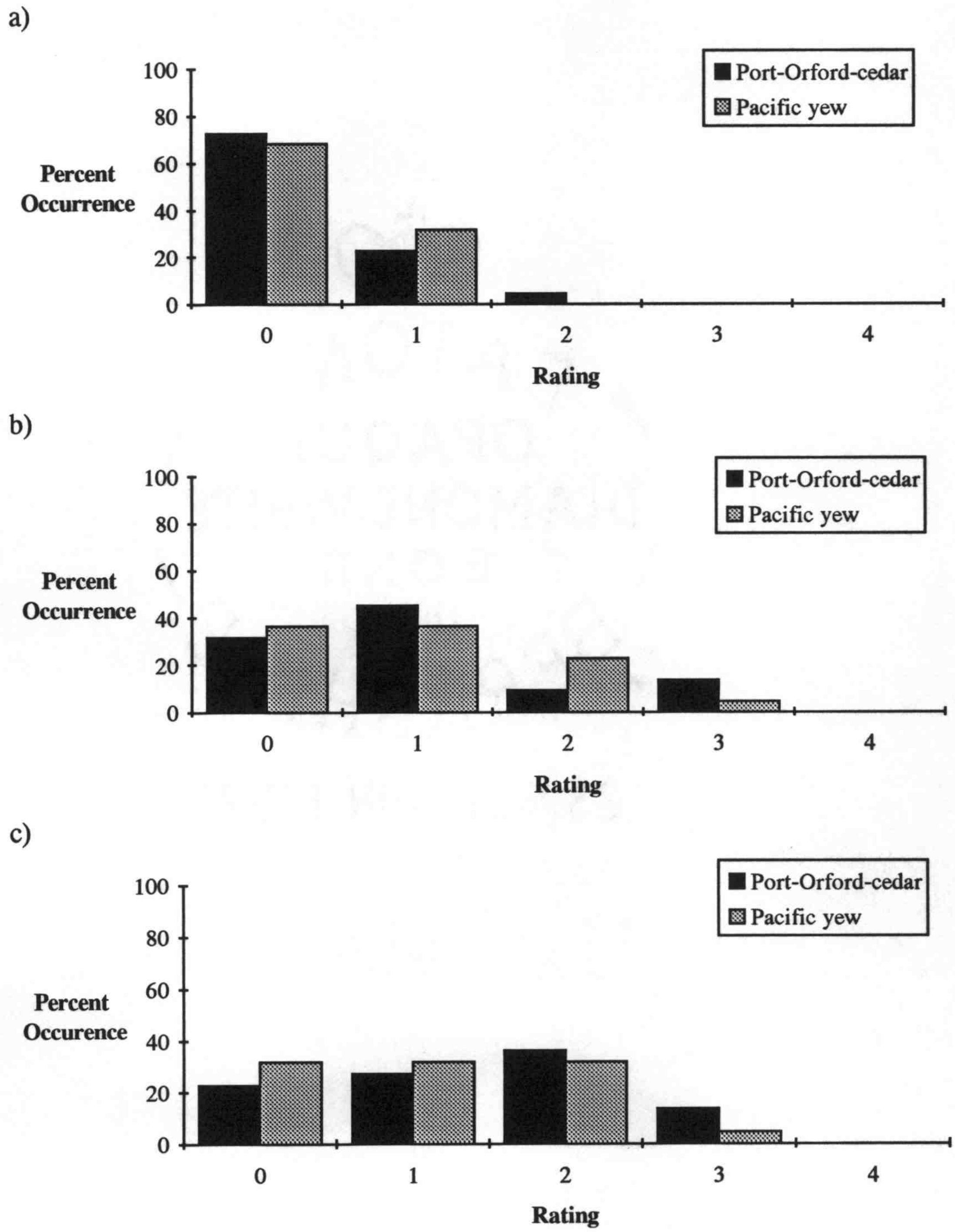


Figure 4a-c. Frequency with which Port-Orford-cedar and Pacific yew rootlets received each rating from 0-4 (0=0-10 zoospores, 1=11-100 zoospores, 2=101-500 zoospores, 3=501-1000 zoospores, 4=>1000 zoospores) at the **region of maturation** at a) 15 minutes, b) 60 minutes, and c) 240 minutes after exposure to zoospores of *P. lateralis*.

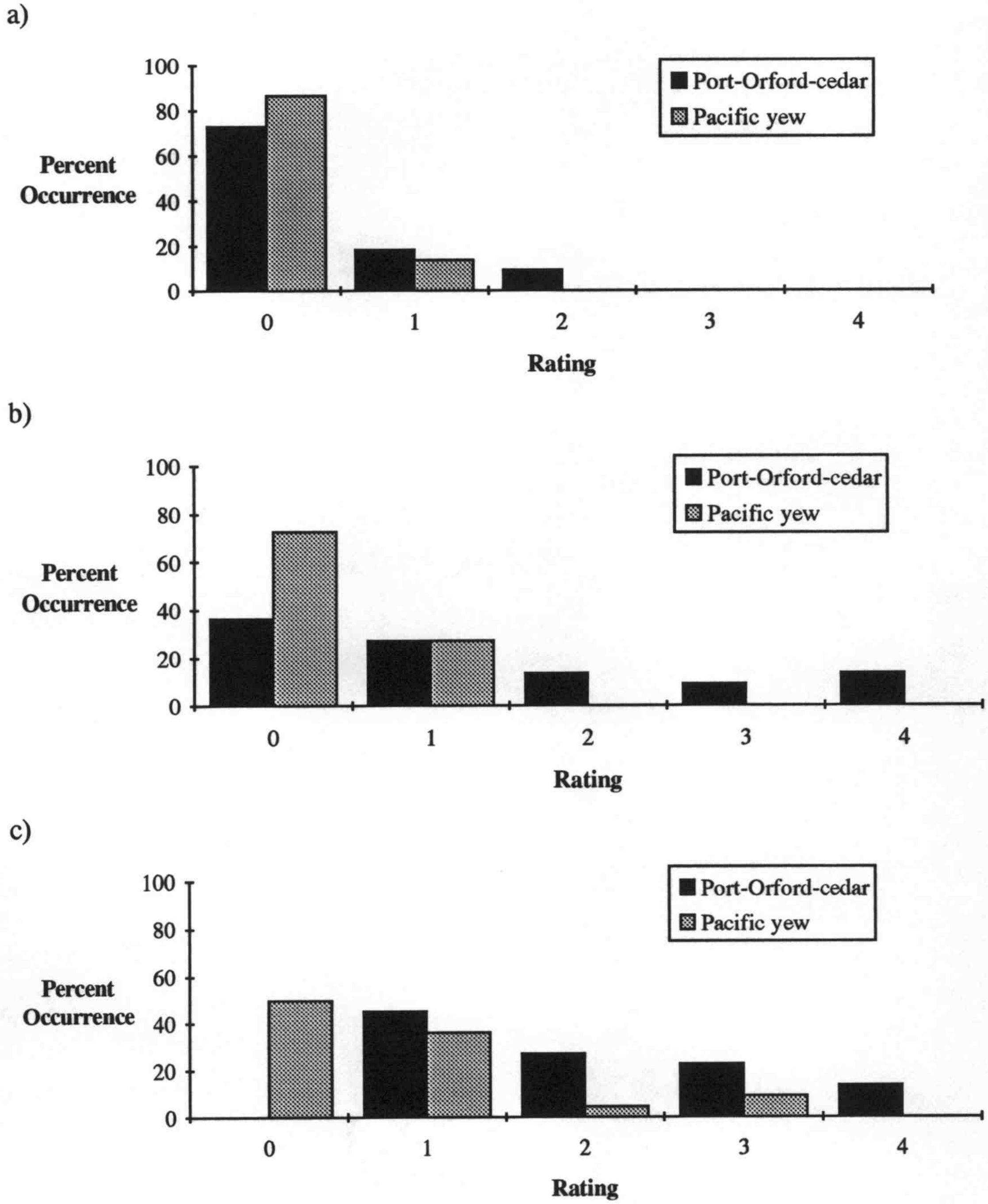
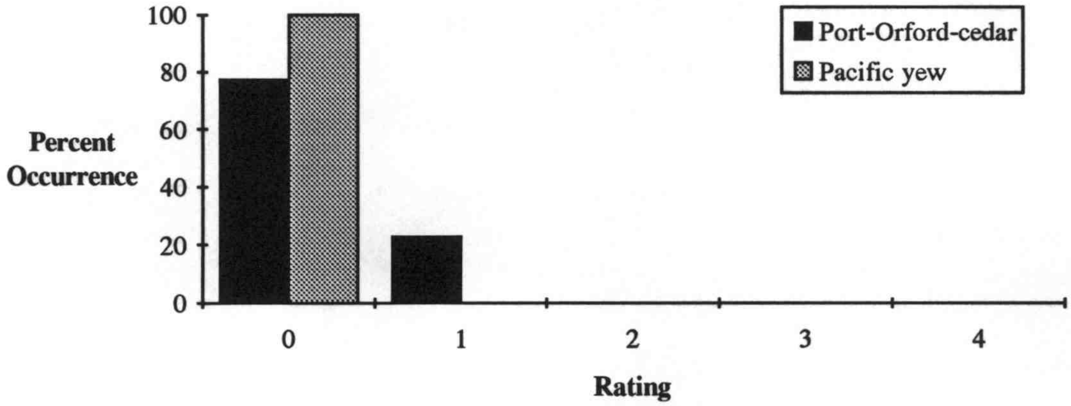
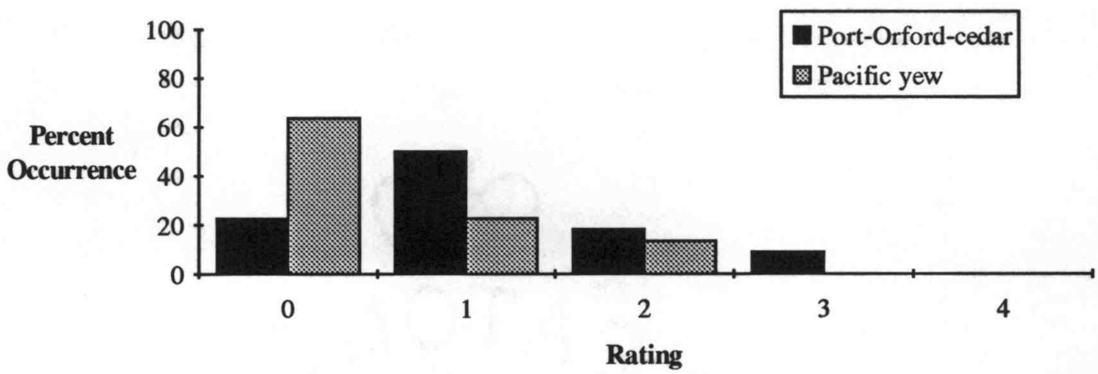


Figure 5a-c. Frequency with which Port-Orford-cedar and Pacific yew rootlets received each rating from 0-4 (0=0-10 zoospores, 1=11-100 zoospores, 2=101-500 zoospores, 3=501-1000 zoospores, 4=>1000 zoospores) at the **zone of elongation** at a) 15 minutes, b) 60 minutes, and c) 240 minutes after exposure to zoospores of *P. lateralis*.

a)



b)



c)

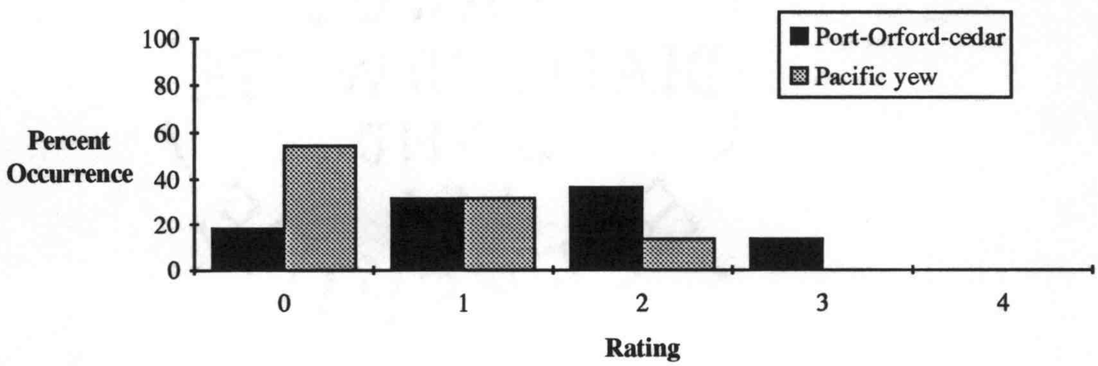


Figure 6a-c. Frequency with which Port-Orford-cedar and Pacific yew rootlets received each rating from 0-4 (0=0-10 zoospores, 1=11-100 zoospores, 2=101-500 zoospores, 3=501-1000 zoospores, 4=>1000 zoospores) at the **root cap** at a) 15 minutes, b) 60 minutes, and c) 240 minutes after exposure to zoospores of *P. lateralis*.

Table 8. Type 3 statistics of regression of poisson distribution of zoospore rating, from 0-4 (0=0-10 zoospores, 1=11-100 zoospores, 2=101-500 zoospores, 3=501-1000 zoospores, 4=>1000 zoospores), comparing differences in amount of zoospore encystment by root segment among Port-Orford-cedar and Pacific yew rootlets (species*rating, in bold) (type 1 results are similar).

	Source of variation	Degrees of Freedom	Chi-Square	Pr > Chi
		15 Minutes		
Root cap	species	1	5.28	0.02
	rating	1	35.12	0.00
	species*rating	1	7.57	**0.00**
Zone of elongation	species	1	2.43	0.11
	rating	2	44.77	0.00
	species*rating	2	3.17	**0.20**
Region of maturation	species	1	1.05	0.30
	rating	2	37.55	0.00
	species*rating	2	1.75	**0.42**
Cut end	species	1	1.32	0.25
	rating	1	52.77	0.00
	species*rating	1	1.41	**0.23**
Total rootlet	species	1	0.00	0.97
	rating	2	5.26	0.07
	species*rating	2	8.45	**0.01**
		60 Minutes		
Root cap	species	1	2.10	0.15
	rating	3	17.95	0.00
	species*rating	3	9.66	**0.02**
Zone of elongation	species	1	8.50	0.00
	rating	4	36.23	0.00
	species*rating	4	16.09	**0.00**
Region of maturation	species	1	0.03	0.86
	rating	3	13.63	0.00
	species*rating	3	2.66	**0.44**
Cut end	species	1	1.17	0.28
	rating	2	11.57	0.00
	species*rating	2	7.85	**0.02**
Total rootlet	species	1	0.91	0.34
	rating	4	18.24	0.00
	species*rating	4	10.24	**0.04**
		240 Minutes		
Root cap	species	1	3.76	0.05
	rating	3	15.55	0.00
	species*rating	3	14.79	**0.00**
Zone of elongation	species	1	0.71	0.40
	rating	3	12.28	0.00
	species*rating	3	21.86	**0.00**
Region of maturation	species	1	0.25	0.61
	rating	3	8.26	0.04
	species*rating	3	1.52	**0.67**
Cut end	species	1	3.36	0.07
	rating	2	17.80	0.00
	species*rating	2	8.40	**0.01**
Total rootlet	species	1	3.28	0.05
	rating	4	18.07	0.00
	species*rating	4	18.96	**0.00**

Table 9. Type 3 statistics of regression of poisson distribution of zoospore rating, from 0-4 (0=0-10 zoospores, 1=11-100 zoospores, 2=101-500 zoospores, 3=501-1000 zoospores, 4=>1000 zoospores), comparing amount of zoospore encystment among rootlet segments (root cap, zone of elongation, region of maturation, and cut end) within Port-Orford-cedar and within Pacific yew (segment*rating, in bold) (type 1 results are similar).

Species	Source of variation	Deg. of Freedom	Chi-Square	Pr>Chi
		15 Minutes		
Port-Orford-cedar	segment	3	2.17	0.54
	rating	2	58.63	0.00
	segment*rating	4	4.79	**0.31**
Pacific yew	segment	3	9.45	0.02
	rating	2	104.78	0.00
	segment*rating	4	17.26	**0.00**
		60 Minutes		
Port-Orford-cedar	segment	3	0.65	0.88
	rating	4	19.08	0.00
	segment*rating	8	3.16	**0.92**
Pacific yew	segment	3	5.21	0.16
	rating	4	61.67	0.00
	segment*rating	8	25.31	**0.00**
		240 Minutes		
Port-Orford-cedar	segment	3	3.40	0.33
	rating	3	13.67	0.00
	segment*rating	8	14.03	**0.08**
Pacific yew	segment	3	4.51	0.21
	rating	3	53.92	0.00
	segment*rating	8	18.14	**0.02**

Stream Survey

A total of 86 dead yew trees were observed at all the stream sites surveyed compared to 1199 total dead Port-Orford-cedar trees. The dead yew comprised 10% of the population while the dead cedar comprised 46% (Table 10). The amount of cedar mortality was consistently and significantly greater than the amount of yew mortality within each drainage ($P \leq 0.05$) (Table 11).

Table 10. Results of a survey to determine the proportions of live and *Phytophthora lateralis*-killed Port-Orford-cedar and Pacific yew within 9 m of stream centers in three *P. lateralis*-infested drainages in southwest Oregon and northwest California.

	Port-Orford-cedar		**Pacific yew**	
Size Class	**Total**	**Dead (%)**	**Total**	**Dead (%)**
		Middle Fork of the Smith River		
≤12 cm DBH	391	50 (13)	324	32 (10)
>12 cm DBH	504	199 (39)	74	13 (18)
Total	895	249 (28)	398	45 (11)
		Coon Creek		
≤12 cm DBH	485	236 (48)	47	15 (32)
>12 cm DBH	344	305 (89)	4	1 (25)
Total	829	541 (65)	51	16 (31)
		Elder Creek		
≤12 cm DBH	421	133 (32)	342	23 (7)
>12 cm DBH	441	276 (62)	46	2 (4)
Total	862	409 (47)	388	25 (6)

Table 11. Type 3 statistics of regression of the binomial distribution of the proportion of dead Pacific yew and Port-Orford-cedar, comparing the number of trees killed by *P. lateralis* per species by stream (type 1 results similar to type 3).

Stream	Source of variation[1]	DF	Chi-Square	Pr>Chi
Middle Fork	species	1	13.7586	0.0002
	size	1	24.2113	0.0000
	species*size	1	4.5070	0.0338
Coon	species	1	11.5831	0.0007
	size	1	1.5991	0.2060
	species*size	1	4.8622	0.0275
Elder	species	1	132.0737	0.0000
	size	1	0.9503	0.3297
	species*size	1	7.5873	0.0059

[1]Species is a comparison of the number of dead POC vs. number of dead Pacific yew.
Size is a comparison of the number of dead trees in size class 1 vs. number of dead trees in size class 2.

On all streams, most living cedar and yew trees were less than 12.4 cm in diameter (Table 10). The greatest number of living POC occurred at the Middle Fork (646), and the least number at Coon Creek (288). The greatest number of living Pacific yew occurred at Elder Creek (363) and the least number at Coon Creek (35 trees). The amount of POC mortality differed significantly among the three drainages ($P \leq 0.05$, Table 12), with the greatest occurring at Coon Creek. The data also suggests that the greatest proportion of dead yew occurred at Coon Creek, and the least at Elder Creek (Table 10), although these differences were not significant.

Table 12. Type 3 statistics for regression of the binomial distribution of the proportion of dead Pacific yew and Port-Orford-cedar, comparing the number of trees killed by *P. lateralis* per stream by species (type 1 results similar to type 3).

Species	**Source of variation**[1]	**DF**	**Chi-Square**	**Pr>Chi**
Port-Orford-cedar	stream	2	356.11	0.00
	size	1	317.76	0.00
	stream*size	2	12.51	0.00
Pacific yew	stream	2	9.96	0.01
	size	1	0.01	0.92
	stream*size	2	2.53	0.28

[1]Stream is the comparison of number of dead trees over the three streams, Elder Creek, Coon Creek, and the Middle Fork of the Smith River.
Size is a comparison of the number of trees in size class 1 vs. size class 2, over three streams.

The Middle Fork of the Smith River, with the largest number of living cedar, had the smallest proportion of dead cedars (249, or 28%), the majority of which occurred in the large size class (size 2). Out of 398 Pacific yew trees at the Middle Fork, 11% of those were dead, the majority of which occurred in the small size class (size 1). Coon Creek was the only surveyed stream with more dead POC's than living (541 out of 819, or 65%), the greatest proportion of which occurred in the large size class. Coon Creek also had the greatest proportion of dead Pacific yew (16 out of 51, or 31%). At Elder Creek, almost half (47%) of the POC were dead, the majority of which were in size class 2. The smallest proportion of dead Pacific yew was observed at this site (25 of 363 trees, or 6%), 92% of which occurred in size class 1.

DISCUSSION

Results of the root inoculations, branch and stem inoculations, zoospore attraction to rootlets, and stream survey all support the original hypothesis that Pacific yew is less susceptible to *P. lateralis* than is POC.

Every method of root inoculation--root dip, chlamydospore, planting alongside an infected POC seedling (InfPOC), and planting in soil amended with infected POC rootlets (InfSoil)--resulted in diseases responses that were consistently higher in the POC seedlings than in any of the three Pacific yew groups.

Past inoculations of Pacific yew have included a preliminary root dip inoculation experiment by the author, and injection of macerated *P. lateralis* mycelium into the soil (DeNitto and Kliejunas 1991). The root dip inoculation was performed on 40 Pacific yew seedlings, and after 27 wk, resulted in no mortality. DeNitto and Kliejunas' method resulted in mortality of half the yews tested. Therefore, in order to determine if a method of inoculation other than the two previous methods would result in mortality of Pacific yew, three treatments were chosen, along with the root dip method, to be included in this study.

The chlamydospore inoculation was chosen to mimic a field situation where primary inoculation occurs via chlamydospores in soil water. The infected cedar root tissue plus soil saturation methods (InfPOC and InfSoil) were also used to mimic field conditions. The water saturation technique was used for two purposes: to encourage zoospore release, and to stress the seedlings and cuttings in a way similar to their situation in most infested drainages. Pacific yew often grows on adjacent banks or in the stream bed itself resulting in a regular saturation of the roots and repeated inoculation with *P. lateralis* via zoospores. The 1991 USFS field survey found that all the confirmed dead yew trees were located near slow moving water or in saturated soils. Furthermore, greenhouse studies show that periodic flooding increased the mortality of pepper plants after inoculation with *P. capsici* chlamydospores (Bowers and Mitchell 1990), and that waterlogging increased the overall proportion of roots infected on *Eucalyptus marginata* seedlings after inoculation with *P. cinnamomi* zoospores (Davison and Tay 1990).

Four measurements were used to test for seedling or cutting susceptibility: percent necrotic root tips, lesion length, percent root mass necrosis, and survival. Based on preliminary inoculations of Pacific yew, the two standard measurements, percent root mass necrosis and survival, were not reliable. Little damage was seen in the preliminary inoculated seedlings, so the additional variables were added. It is recommended, however, to disregard the measurement of necrotic root tips in future inoculation studies. Counting root tips is time-consuming, and the fact that the control seedlings and cuttings had necrotic root tips suggests that other factors aside from *P. lateralis* are contributing to tip necrosis, including other fungi and/or natural root pruning, which would confound results.

Out of the four inoculation methods used, the root dip method is recommended for future studies for ease of labor, time, and inducement of infection and mortality on both species. Mortality of Pacific yew and POC was highest with this method, a result that may be attributable to several reasons. A definitive point source of inoculation occurred at every root end. The root mass of each seedling had been cut to an even length and exposed to the inoculum which would result not only in multiple infections for the plant, but also in multiple infections at each root end. In addition, wounding of the root tips may have been necessary for an infection court. Deliberate root wounding of either species did not occur for any other inoculation method.

Branch and stem inoculation results supported root inoculation results, similar to studies by Hansen and Hamm (1989) with POC and *P. lateralis*, by Browne and Mircetich (1993) with apple and *P. cactorum*, *P. cambivora*, and *P. cryptogea*, and by Afek and Sztejnberg (1990) with citrus stems and *P. citrophthora*, although these studies used branches and seedlings that had originated from the same parent trees. Lesion growth of *P. lateralis* in POC was much faster than in Pacific yew in both the tree branches and the intact seedling stems.

Results of the zoospore exposure to rootlets suggest that far fewer zoospores will encyst on Pacific yew rootlets than on cedar rootlets, given the same approximate inoculum concentration and time of exposure. The primary location of zoospore encystment on the POC rootlets was consistent with prior studies. Zentmeyer (1980) found that *P. cinnamomi* zoospores were more attracted to the region of elongation in small feeder roots of avocado than to the root tip or to the zone of differentiation. Zoospores encysted at different distances from the root tip as if in response to a concentration gradient of some stimulatory chemical exuding from the root, such as sugars, amino acids, ethanol, or various cationic molecules. Surprisingly, this area of the rootlet represented one of the smallest surface areas of the four segments.

Based on the result that mortality of Pacific yew was highest in the root dip method of inoculation, possibly due to root wounding, one may expect that zoospores would be if not mostly, at least highly attracted to the cut ends of the rootlets. This segment, on both hosts, however, was the least attractive to zoospores. Zoospores did encyst on this area, and perhaps the amount of zoospores is a primary factor in infection, but the amount of spores that germinate and penetrate successfully. Hyphae may have a greater chance of colonizing exposed phloem tissue than penetrating epidermal and cortical layers. Zoospores on Pacific yew rootlets most commonly encysted along the region of maturation, and were most heavily concentrated on the root hairs, areas that are least likely to be producing chemical defense compounds. The region of maturation represented the largest surface area of the four rootlet segments. Although it wasn't analyzed statistically, the data suggest that the amount of zoospores that encysted on the relatively smaller zone of elongation observed on the POC rootlets was in fact greater than the amount of zoospores that encysted on the larger region of maturation observed on the Pacific yew rootlets.

The field survey of infested drainages further supports the original hypothesis that Pacific yew is a less susceptible host of *P. lateralis* than POC. The proportion of dead POC in each drainage was significantly greater than the proportion of dead Pacific yew. The overall mortality of Pacific yew, however, is much greater than previously reported, although it is sporadic along stream banks while POC mortality is almost uniform along stream banks. Mortality of Pacific yew trees in infested drainages is most likely due to a combination of several factors, including genetics, extent and duration of soil saturation, *P. lateralis* inoculum level, and/or other environmental conditions.

The variation in response to *P. lateralis* expressed in the Pacific yew root, branch, and stem inoculations may suggest a genetic difference in susceptibility within the host. Experimental and environmental factors, however, limit the scope of such a conclusion. Each root inoculation method was performed on two independent occasions, and the branch and stem inoculations on three independent occasions. As a result, the amount of inoculum each plant received may have varied, as evidenced, for example, in the chlamydospore inoculation method. Fungal recovery for this method was low, suggesting that the inoculum level, or the rate of spore germination, differed between inoculation dates. Additionally, although the greenhouse was monitored for light and temperature conditions, slight differences, such as an increase in temperature, may have occurred between inoculation dates that would have affected inoculum characteristics or the response of Pacific yew. Results of the POC inoculations, however, counter the above arguments and lend support to the hypothesis that Pacific yew varies in its susceptibility. No differences were detected between the first and second POC seedling inoculation groups, nor between the POC branch and stem inoculations.

Both POC and Pacific yew seem to have a moderate level of genetic diversity. Pacific yew trees tested from three geographic regions of British Columbia were shown to have slightly higher levels of genetic diversity than associated temperate zone species, and lower than species with widespread geographic distribution (El-Kassaby and Yanchuk 1994). Similar results were revealed in a U.S. Forest Service study (Vance 1992, unpublished) from 54 populations throughout the range of Pacific yew. Researchers found a moderate amount of genetic variation with an average observed population heterozygosity of 0.171, although no significant differences were seen among populations from northwest California and the Siskiyou Mountains. Observed diversity of POC seems to be slightly lower than Pacific yew. Variation in electrophoretically detectable loci of nine disjunct populations of POC in northern California was moderately high, with an observed heterozygosity of 0.13 (Millar 1991).

The results of the seedling and cutting inoculations, branch inoculations, zoospore attraction, and field observations all show that Pacific yew is much less susceptible than POC to *P. lateralis*. However, the fact that *P. lateralis* was isolated from a majority of the inoculated Pacific yew seedlings and cuttings should not be overlooked. Pacific yew in infested drainages could actually be increasing the survival and concentration of *P. lateralis* while surviving exposure to the pathogen. Such conditions would hinder the management of POC. In areas where the two hosts occur together, an increased risk of mortality of POC may occur over a longer period of time due to the presence of Pacific yew. Managers, therefore, should be especially wary in areas where the two host species occur together. When healthy cedar is removed as a means of eradicating *P. lateralis* and preventing future infections, the removal of all healthy Pacific yew as well should be considered.

As of today, prevention of spread is the primary means of control of *P. lateralis* root rot in the native range of POC. According to the Inter-Regional Coordinating Plan, established in 1988 by representatives from the USFS, BLM, and research (Barker and Torrence 1988), Forest Service Regions 5 and 6 have agreed to establish check stations for washing truck and logging equipment free of soil, build road berms, limit logging in sensitive areas to the dry season, close roads during the wet season, monitor, educate the public, and conduct research. Effectiveness of these control strategies and their link to Pacific yew has yet to be determined.

Results of this thesis suggest that *P. lateralis* will have limited impact on the native population of Pacific yew over its entire range. Unusual mortality of Pacific yew has not been observed anywhere outside the range of POC (Greenup, pers. comm). The slow growing yew is associated with a variety of conifer and hardwood tree species outside the range of POC. It grows on several different soil types and altitudinal zones, from the southern tip of southeast Alaska to Calaveras County, California, and is usually found scattered on upland sites or sporadically in riparian areas (Bolsinger and Jaramillo 1990). Stands are most dense and abundant, however, in southern Oregon and in the northern Rocky Mountains (Daubenmire and Daubenmire 1968 and Franklin and Dyrness 1972). In the native range of POC, however, *P. lateralis* does have the potential to alter the population of Pacific yew at specific micro-sites. The 1991 USFS survey, which covered most of the *P. lateralis*-infested portion of the cedar's native range, found that most of the dead Pacific yew were located in heavily infested areas and in saturated soils. The areas of greatest concern are those infested sites where the soil is saturated much of the year. Results of this thesis also suggest, however, that Pacific yew varies in its susceptibility to the pathogen. As a result, we may conclude that even in areas of high risk for infection, mortality will continue to be sporadic and low.

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Chapter 3. Site Factors Influencing Mortality of Pacific Yew in *Phytophthora lateralis*-Infested Drainages in Southwest Oregon and Northwest California

INTRODUCTION

Pacific yew (*Taxus brevifolia* Nutt.) was reported as a new host for *Phytophthora lateralis* in 1991 (DeNitto and Kliejunas). In the summer of that same year, U.S. Forest Service (USFS) field crews successfully isolated *P. lateralis* from 17 dead Pacific yew trees after surveying all the major *P. lateralis*-infested drainages in southwest Oregon and northwest California (DeNitto USDA Forest Service, pers. comm.). According to results of the survey, mortality appeared to occur only in areas with high inoculum levels and long-term soil saturation, although this has not been closely examined.

Phytophthora lateralis is known to be an aggressive pathogen on Port-Orford-cedar (POC) (*Chamaecyparis lawsoniana* (A. Murr.) Parl.), previously thought to be the only susceptible host (Roth, Trione, and Ruhmann 1957). Maps of POC mortality reveal extensive portions of its natural range, in southwest Oregon and northwest California, are infested (Greenup 1992). Previous research has shown that dead cedar are associated with drainages, ditches, and along roads (Hansen, Final Report on Port-Orford-cedar Evaluation, 1992; Roth, Harvey, and Kliejunas 1987; Roth, Trione, and Ruhmann 1957). Remaining live POC growing in infested drainages and along infested road systems seem to have escaped infection largely by chance, as resistance in the field has not been documented. Pacific yew mortality, however, is much more sporadic along these same drainages, and rare along roads.

The Pacific yews that were recorded as positive for *P. lateralis* in the 1991 field survey were confirmed through isolation by plating infected tissue onto selective media, a laborious process that is not always successful. Several drawbacks limit the application of direct plating for diagnostic purposes. First, the tree in question must have been recently killed in order to identify the region of active fungal growth. *Phytophthora lateralis* forms chlamydospores in host tissue once the tree dies, and fungal growth from these structures is not consistently successful on media. Most of the dead yew trees in these infested drainages have been dead several years. Second, the infected tissue collected must be plated onto media immediately. And third, after plating tissue, results are not instantaneous, and contamination can hinder the identification process. Using a serological technique such as ELISA (enzyme-linked immunosorbent assay) can be a far more efficient method in verifying cause of death. Results are obtained on the day of the test, determined either visually, or with the aid of an ELISA plate reader. In addition, commercial ELISA tests are available that can be used on-site, providing diagnostic results within minutes.

The overall objective of this study was to determine if Pacific yew mortality is associated with heavily infested areas whose soils are saturated much of the year. A commercially available ELISA kit was used for determining the presence of *P. lateralis* in the field study plot trees. Prior to its use, the efficacy of the kit was established by comparing results of visibly diseased trees to negative controls. In the summer and fall of 1993, temporary field plots were established in infested drainages in southwest Oregon and northwest California to determine if site differences existed between Pacific yew trees killed by *P. lateralis* and adjacent, live Pacific yew trees. Our hypothesis was that dead Pacific yew are more closely associated with areas of saturated soils and high inoculum potential than live Pacific yew.

METHODS

Efficacy of ELISA

Sampling Method

Phloem tissue was sampled at the root collar where *P. lateralis* would normally occur, from 13 dead and dying Pacific yew and from 13 dead and dying POC trees. Negative control samples were collected from those same trees 1.8 m up the main stem, where the pathogen should not occur. Trees were located along Elder Creek, a *P. lateralis*-infested drainage on the Illinois Valley Ranger District in the Siskiyou National Forest. Known infected and known uninfected tissues were also included in the study. Known uninfected tissue was extracted from the base of one Pacific yew tree that had died due to means other than *P. lateralis* (probably drought) and from one healthy POC, both in the same drainage. Known infected tissue consisted of visibly symptomatic root tissue taken from two seedlings of each species that had been previously inoculated with *P. lateralis*.

Each tree was sampled by removing at least three 4 x 5 cm inner and outer bark chips with a hatchet. Tissue sampled from infected seedlings consisted of 1-2 cm long, visibly infected root segments. All samples were individually bagged, labeled, and refrigerated, and the ELISA test was performed within 24 h of sample collection.

ELISA Procedure

A commercial 96-well double-antibody ELISA test was used for this study (Neogen, Corp. Lansing, MI), and all methods were as recommended in the package directions. Each sample was prepared by first grinding the tissue on a 2.5 x 5 cm section of 180 grit sandpaper, and then soaking and vortexing the sandpaper in an extraction solution for 20 sec. The sandpaper was removed, and 100 μl of each sample was then added to each of two individual antibody-coated wells in the 96-well microtiter plate, along with the provided positive and negative controls. After a 10 min incubation period, all wells were washed with a detergent solution, followed by the addition of 100 μl of the antibody-enzyme conjugate solution to each well. The plate was then agitated for 10 min, washed, and 100 μl of the substrate solution was added to each well. The plate was agitated and incubated for an additional 10 min, and 50 μl of a stop solution was added. Light absorption of each well at a wavelength of 405 nm was determined using a microplate reader (V-Max Kinetic, Molecular Devises Corp, Menlo Park, CA). A sample was considered positive for *P. lateralis* if the absorption reading was more than three times the value of the provided negative control.

A Student's t-test was used to compare base and bole sample readings within both Pacific yew and POC using Statistical Analysis Systems version 6.08 (SAS Institute, Cary, NC).

Field Plots

Experimental Design

In July-September of 1993, field plots were established in southwest Oregon and northwest California where Pacific yew trees had been killed by *P. lateralis*, as determined through a 1991 U.S. Forest Service field survey. Plots were located in a total of six infested drainages, including the upper reaches of the Middle Fork of the Smith River, an unnamed tributary of Coon Creek, upper Monkey Creek, and Baker Flat, all located on the Gasquet Ranger District of Six Rivers National Forest in northern California, and lower Elder Creek and Panther Ridge, located on the Illinois Valley Ranger District and Powers Ranger District, respectively, in Siskiyou National Forest, southwest Oregon (Figure 7). Stream descriptions and locations are shown on Table 13.

Table 13. Locations and descriptions of Pacific yew study sites.

Stream	Legal description	No. plots	% Gradient[1]	Date of *P. lateralis* introduction	Source of introduction
Middle Fork	T17N, R4E, S6	10	5	1986	TSI[2]
Coon	T16N, R2E, S21	13	10	1984	unknown
Monkey	T18N, R3E, S12, 13	3	10	1987	mining
Baker Flat	T18N, R3E, S2	1	5	1983	road grading
Elder	T40S, R7W, S19, 20	17	10	mid-1970's	unknown
Panther Ridge	T32S, R11W, S26	3	10	unknown	unknown

[1]Average gradient of the stream channel where plots occurred, measured with a clinometer.
[2]TSI=Timber Stand Improvement (pre-commercial thinning)

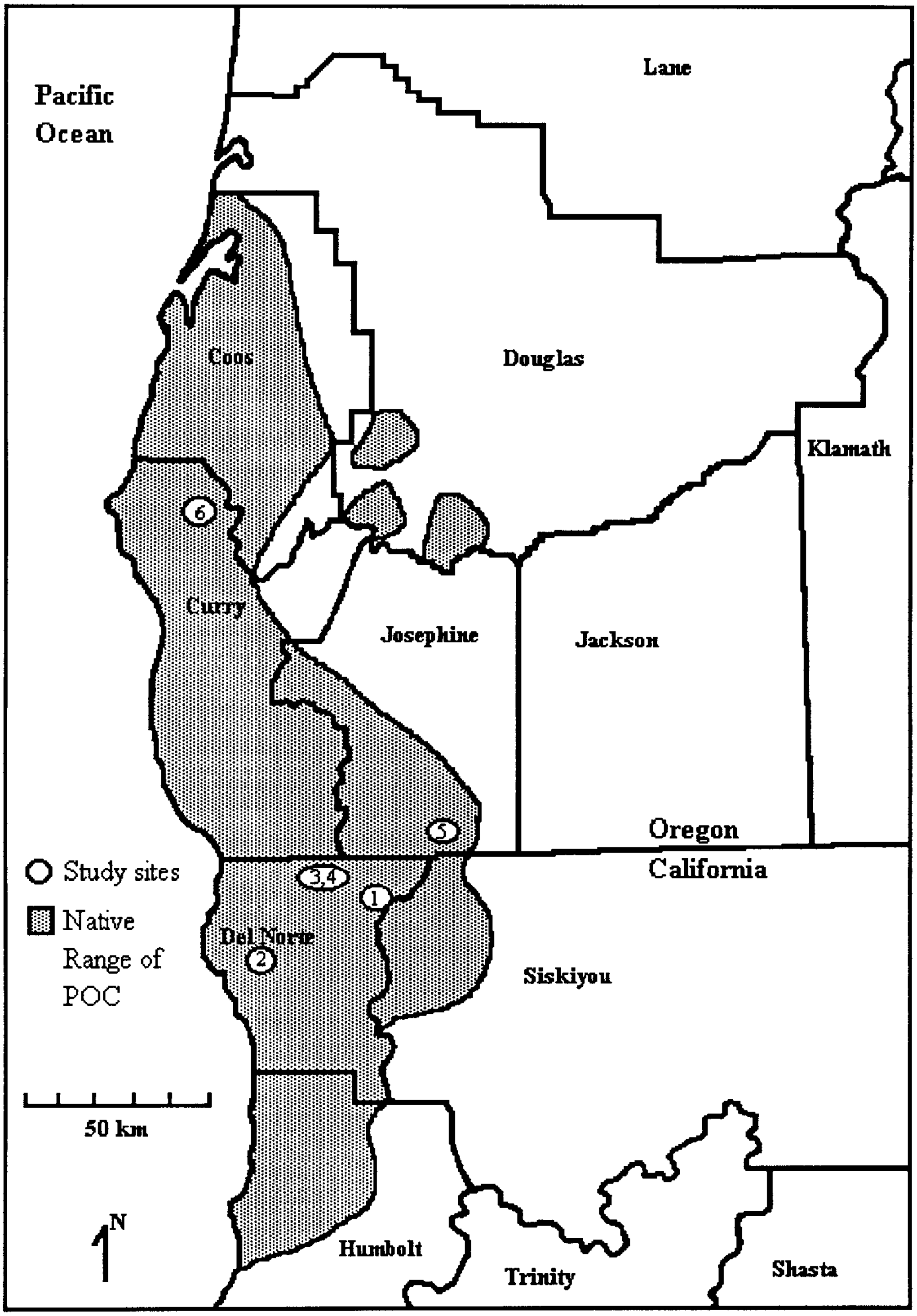

Figure 7. Study site locations within the native range of Port Orford cedar. 1--Middle Fork, 2--Coon Creek, 3--Baker Flat, 4--Monkey Creek, 5--Elder Creek, and 6--Panther Ridge.

Each plot comprised two or three 0.025 ha subplots (9-m-radius). The first subplot was centered on a dead yew, the second on a nearby live yew, comparably situated, and the third, on a live yew uphill from the stream. Plots at the Middle Fork, Coon Creek, and Elder Creek were located within the survey segments discussed in Chapter 2 of this thesis. Plots at the remaining three locations were established only in areas where the USFS had previously identified dead yew trees, aside from Panther Ridge, where two additional plots were included. Plots were established based on the presence of a suitable dead yew tree, which was chosen based on three criteria: 1) bark present at base for sample collection and use in ELISA; 2) *Phytophthora lateralis* probable cause of death; and 3) a live yew tree present at least 9 m and not more than 15 m from the dead yew. At 90% of the plots, only one live yew was nearby. However, if more than one live yew was present within the specified distances, then subplots were established around the two closest trees, and the measurements averaged between the two. A second live yew, if present, was chosen uphill and away from the stream. An uphill Pacific yew was included in the study only if a) its roots had little chance of contacting the stream water (at least 9 meters away from the high water mark), and b) it could be seen from the water's edge and from the chosen dead tree (to determine distances with a rangefinder). At 85% of the plots, only one uphill yew tree meeting these criteria was observed. If more than one tree was available, then the closest suitable live tree to the chosen dead tree was selected.

Data Collection and Analysis

Data recorded at each subplot included: height and diameter at breast height (DBH) of the subplot center tree, percent canopy cover (estimated visually), percent slope from the subplot center to the water's edge (measured with a clinometer), and slope distance from subplot center tree to the stream's high and low water marks (measured with a digital rangefinder). The high water mark was defined as the location where growth of vegetation began, and was clearly visible at all subplots. The low water mark was defined as the edge of the stream at the time of sampling. Finally, the diameter of all live and *P. lateralis*-killed POC (as determined by visual inspection) on the subplot was recorded in one of five DBH classes:

1=seedling (up to 1.35 m in height)
2=1.25-12.4 cm, mean=6.8 cm
3=12.5 cm-22.6 cm, mean=17.5 cm
4=22.7-53.1, mean=37.9 cm
5=53.2+, "mean"=63.5 cm

The DBH data was then converted to two basal area values for each subplot: live, and *P. lateralis*-killed, POC. To do so, the diameter rating of each POC was expressed as the mean diameter representing that class, as shown above. The seedlings were disregarded, and the diameters were converted to subplot basal areas. The basal area of *P. lateralis*-killed trees was treated as a measure of the amount of inoculum available in each subplot.

Finally, inner and outer bark chips were collected from the root collar of each dead yew subplot center tree for confirmation of *P. lateralis* via two methods. Hamm and Hansen's (1984) double-cup baiting technique was used first (see Chapter 2, Root Inoculations, Data collection and Recovery of *P. lateralis*), followed by an ELISA test, performed as described previously.

Analysis was performed comparing the dead/live stream-side pairs only, using a generalized linear model of the binomial distribution of dead trees to total trees, blocked by subplot number, using Statistical Analysis Systems version 6.08 (SAS Institute, Cary, NC). Distance to high and low water and both live and dead POC basal areas were log-transformed to achieve normality and equal variance.

RESULTS

Efficacy of ELISA

Absorption readings (405 nm) for the ELISA analysis of Pacific yew and POC samples are shown in Figures 8 and 9. Results indicate that samples taken from the base of dead or dying trees that were suspected to be infected by *P. lateralis* were positive, while the control samples taken from 1.8 m up the main stem on the same trees were negative. The mean absorption reading for the Pacific yew base samples (2.058), was significantly greater ($P \leq 0.05$) than the mean bole sample reading (0.176). Additionally, the mean absorption reading for the POC base samples (0.733) was also significantly greater ($P \leq 0.05$) than the mean bole sample reading (0.066). The known infected tissue taken from the roots of the previously inoculated Pacific yew and POC seedlings exhibited positive readings (samples 15 and 16, Figures 8 and 9), and the known uninfected tissue taken from the base of the Pacific yew tree that was killed due to means other than *Phytophthora*, and from the healthy POC tree exhibited negative readings (sample 14, Figures 8 and 9). For the purposes of this study, the ELISA test sufficed for determining the presence of *Phytophthora* in Pacific yew.

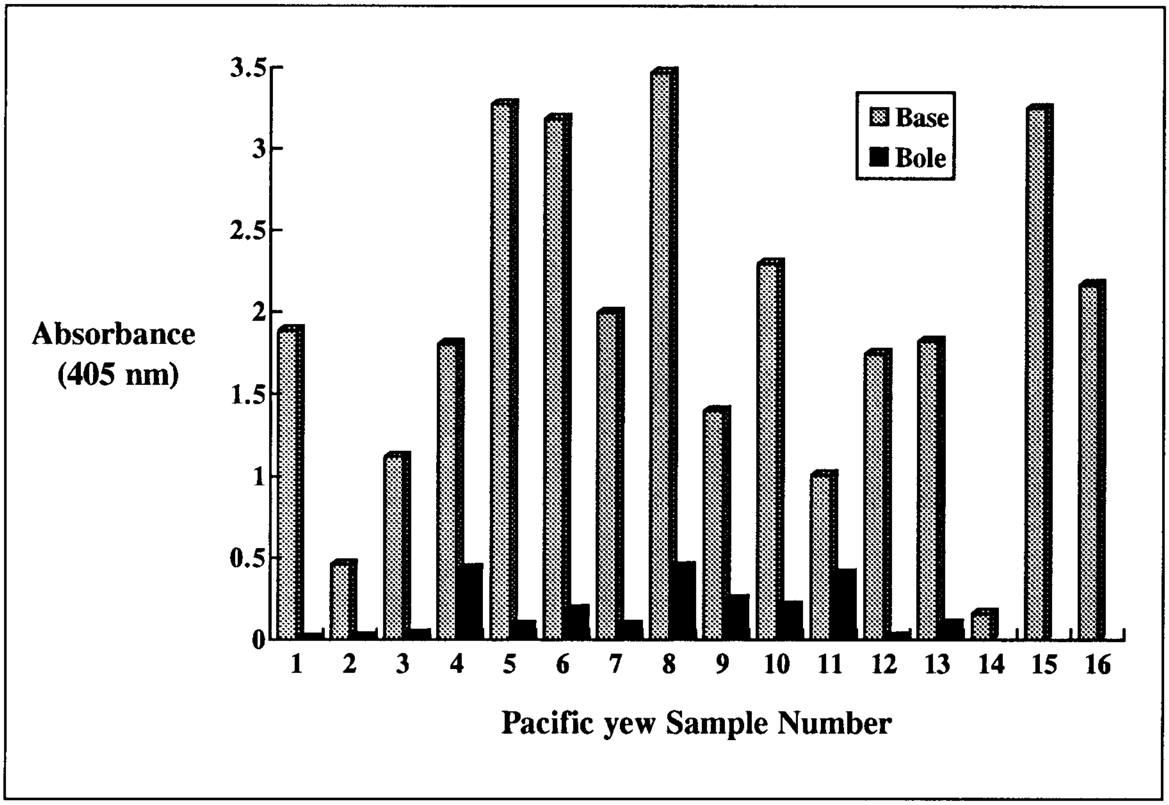

Figure 8. Absorbance readings of samples taken from the base and bole of dead **Pacific yew** trees located in a *P. lateralis*-infested drainage in southwest Oregon. Sample number 14 is from a known uninfected yew tree, and sample numbers 15 and 16 are from known infected seedlings inoculated with *P. lateralis*. (Kit provided negative control was -0.033.)[1]

[1]Sample values are means of two replicate wells.

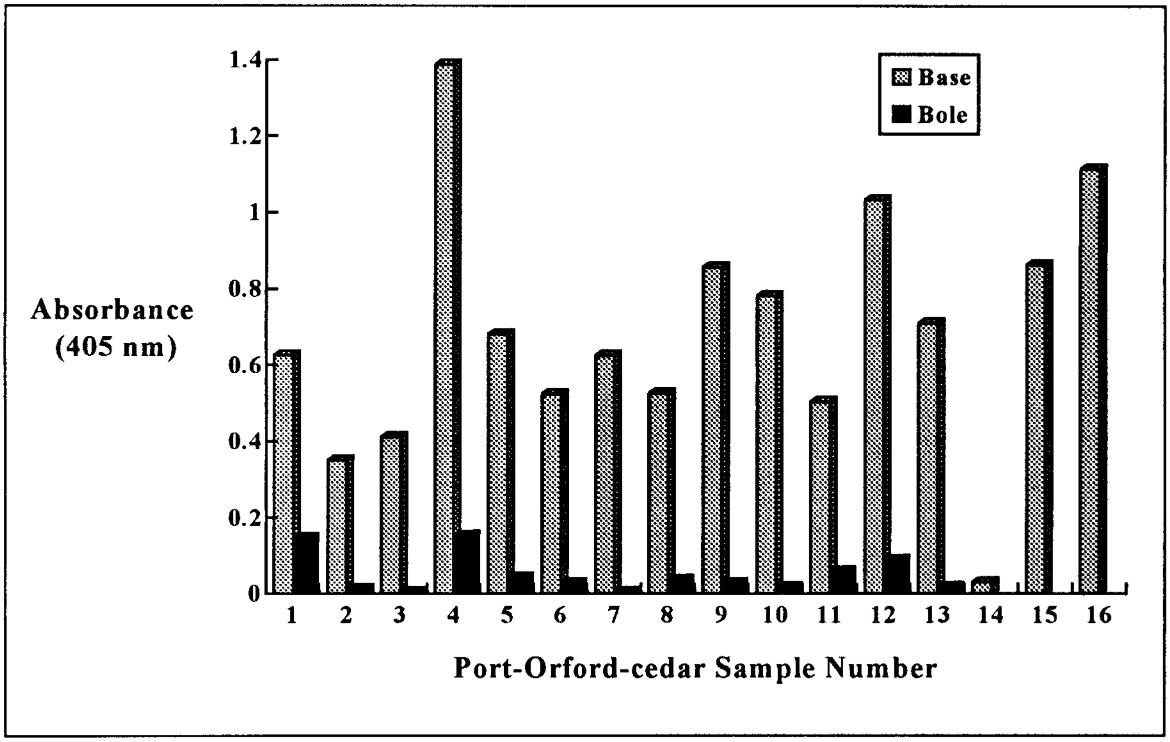

Figure 9. Absorbance readings of samples taken from the base and the bole of dead **Port-Orford-cedar** trees in a *P. lateralis*-infested drainage in southwest Oregon. Sample number 14 is from a known uninfected tree, and sample numbers 15 and 16 are from known infected seedlings inoculated with *P. lateralis*. (Kit provided negative control was -0.019.)[1]

[1]Sample values are means of two replicate wells.

Field Plots

A total of 47 stream-side plots were established. Live uphill yew trees were available on 36 of these plots. The distance to high and low water averaged 3.2 m and 4.5 m, respectively, for the dead tree subplots, 3.7 m and 4.9 m, respectively, for the live stream-side subplots, and 14.8 m and 15.9 m, respectively, for the live uphill subplots. The basal area of *P. lateralis*-killed POC averaged 0.51 m^2 on the dead yew subplots, 0.47 m^2 on the stream-side live yew subplots, and 0.01 m^2 on the uphill live yew subplots (Table 14).

Table 14. Means and standard deviations (in parentheses) for parameters measured at 47 paired-tree Pacific yew subplots in *Phytophthora lateralis*-infested drainages in southwest Oregon and northwest California.

Subplot Center	**Height (m)**	**DBH (cm)**	**Slope (%)**	**Canopy Cover (%)**	**Distance to high water (m)**	**Distance to low water (m)**	**BA (m^2) of dead POC**	**BA (m2) of live POC**
Dead	7.8 (1.9)	14.9 (3.9)	32 (18.3)	42 (23.9)	3.2 (6.8)	4.5 (4.4)	0.52 (0.47)	0.20 (0.21)
Live-stream	8.1 (2.1)	15.2 (5.0)	35 (23.1)	45 (25.3)	3.7 (5.9)	4.9 (4.8)	0.47 (0.43)	0.21 (0.25)
Live-uphill	7.9 (1.8)	14.2 (4.9)	38 (21.8)	47 (29.5)	14.8 (6.2)	15.9 (6.7)	0.01 (0.02)	0.02 (0.06)

Table 15. Type 1 and type 3 statistics showing the significant variables included in the final regression model comparing dead Pacific yew subplots to live Pacific yew subplots in infested drainages in southwest Oregon and northwest California.

		Chi-square		**Pr>Chi**	
Source of Variation	**DF**	**Type 1**	**Type 3**	**Type 1**	**Type 3**
Pair	46	0.00	25.05	1.00	0.99
slope	1	4.14	6.49	0.04	0.01
high water	1	14.04	18.29	0.00	0.00
slope*high water	1	6.19	6.85	0.01	0.01
cover	1	8.09	8.09	0.00	0.00

No significant differences were detected for height, DBH, or distance to low water between the stream-side pair of yews, nor for basal area of *P. lateralis*-killed POC. Percent canopy cover was significantly greater on the live stream-side yew subplots than on the dead yew subplots ($P \leq 0.05$, Table 15).

An important aspect to examine at sites where *P. lateralis* mortality may occur is the combination of distance to water's edge and slope. Roots of trees that are far from the water's edge may be exposed to stream water below ground, especially during flood periods, if the terrain is level to nearly level. This case is shown by the model, where the interaction of slope and distance to high water accounts for a significant amount of variation ($P \leq 0.05$, Table 15). One could infer, then, that close proximity to infested water on flat slopes may increase the probability of mortality of Pacific yew.

The significant data detected in the model are provided on a plot-by-plot and stream-by-stream basis in Tables 16a-d, the first three tables representing Coon Creek, the Middle Fork, and Elder Creek, and the last table, a combination of the remaining three stream locations. Examination of results by stream reveals that the basal area of dead POC between the dead yew and live stream-side yew subplots is similar at Coon Creek and Elder Creek, and greater on the dead yew subplots at the Middle Fork and at the remaining locations. Additionally, distance to high water is also similar for the stream-side subplots at Coon Creek and Elder Creek, while at the Middle Fork and at all other locations, the dead yews were closer to the water than the live yews. By stream, the dead yew subplots were always on nearly level slopes, the greatest frequency of which occurred at the Coon Creek plots.

On a plot-by plot basis, the data support the overall statistical results. On 33 of the 47 plots, the slope of the dead yew subplot was either less than or equal to slope of the live stream-side subplot. On 34 plots, the dead yew was either closer to or equal distance away from the high water mark than the live stream-side yew. And of all the Pacific yew subplot trees that occurred immediately adjacent to the water's edge (distance=0.0 m), 17 were dead, and 6 were live. On 13 plots, the dead yew was both closer to high water and on a gentler slope than the live stream-side yew, while the reverse was true on only 4 plots. Although basal area of dead POC was not a significant measure, on 30 of the 47 plots, basal area of dead POC on the dead tree subplot was greater than or equal to basal area of dead POC on the live stream-side subplot. And finally, on 6 plots, the dead yew was closer to high water, on a gentler slope and had a greater basal area of dead POC than the live stream-side yew, while the reverse was true on only 3 plots.

Data for the live uphill yew subplots was not included in the analysis, however several trends were evident. Basal area of live POC and basal area of *P. lateralis*-killed POC were far less on every live uphill yew subplot than on either the dead yew or live stream-side yew subplots. Canopy cover at these subplots, however, was greater than at either of the stream-side Pacific yew subplots, attributable to tree species other than POC.

Table 16. Site measurements recorded on all Pacific yew plots, by subplot, with means for each drainage.

Table 16. **Coon Creek**

	Percent Slope			Slope distance to high water (m)			Basal area of dead POC			Basal area of live POC			Percent Canopy Cover		
Plot[1]	**dead[3]**	**live-stream**	**live-uphill**	**dead**	**live-stream**	**live-uphill**	**dead**	**live-stream**	**live-uphill**	**dead**	**live-stream**	**live-uphill**	**dead**	**live-stream**	**live-uphill**
1[2] +	62	33	71	0.00	1.51	10.23	0.26	0.31	0.00	0.02	0.04	0.00	10	10	75
3 +	0	58	74	0.45	0.92	20.70	0.60	0.90	0.00	0.05	0.07	0.00	20	20	5
4 +	62	87	90	0.91	0.94	8.10	1.02	1.04	0.00	0.05	0.07	0.00	10	10	30
5 +	51	51	60	1.80	1.21	6.92	0.20	0.31	0.00	0.04	0.05	0.01	15	15	50
6 +	39	40	90	0.30	0.95	10.22	0.39	0.30	0.00	0.10	0.07	0.01	20	20	80
7 +	32	74	42	1.21	1.50	9.00	0.57	0.90	0.00	0.06	0.03	0.00	30	60	55
8 +	30	48	-----	1.23	2.10	-----	1.08	0.63	------	0.07	0.09	-----	10	90	-----
19 +	0	0	8	4.82	7.54	19.53	1.04	0.80	0.00	0.04	0.10	0.00	35	35	40
20 -	0	44	25	14.41	12.92	18.62	1.68	1.75	0.00	0.10	0.05	0.00	25	25	50
21 +	40	44	-----	0.00	0.00	-----	1.08	0.74	-----	0.16	0.49	-----	30	30	-----
22 +	36	59	60	0.00	0.65	13.81	0.28	0.60	0.00	0.13	0.00	0.00	25	25	40
23 +	21	46	30	0.00	0.96	25.50	0.63	0.13	0.00	0.26	0.15	0.05	40	40	90
24 +	24	14	-----	0.00	0.00	-----	0.20	0.28	-----	0.07	0.05	-----	30	70	-----
Means	33.1	43.9	53.6	1.9	2.4	14.2	0.68	0.66	0.00	0.09	0.10	0.01	23.1	34.6	51.5

[1]Results of ELISA readings of the dead yews are provided alongside the plot number, either as positive (+), or negative (-).
[2]The live, streamside subplot data is an average of two live yew subplots.
[3]Pacific yew subplots were centered on a dead yew, a live, streamside yew, and a live, uphill yew.

Table 16, continued. **Middle Fork of the Smith River**

	Percent Slope			Slope dististance to high water (m)			Basal area of dead POC			Basal area of live POC			Percent Canopy Cover		
Plot	**dead**	**live-stream**	**live-uphill**	**dead**	**live-stream**	**live-uphill**	**dead**	**live-stream**	**live-uphill**	**dead**	**live-stream**	**live-uphill**	**dead**	**live-stream**	**live-uphill**
9 +	10	17	30	2.7	4.8	10.5	1.16	0.16	0.00	0.03	0.00	0.00	30	30	15
10 -	15	15	28	0.0	1.2	16.8	0.51	0.25	0.00	0.08	0.05	0.03	55	55	10
11[2] +	15	35	20	2.1	6.9	16.8	0.24	0.91	0.05	0.46	0.37	0.00	20	22	30
12[2] +	18	11	-----	0.0	3.0	-----	0.69	0.68	-----	0.11	0.37	-----	30	25	-----
13[2] -	17	14	8	2.4	1.8	9.6	0.28	0.91	0.01	0.23	0.26	0.05	90	90	90
14 +	13	13	7	1.2	3.3	18.0	1.37	1.07	0.00	0.11	0.03	0.00	15	15	10
15[2] +	24	24	7	4.2	8.1	38.4	1.16	0.99	0.15	0.16	0.11	0.05	75	75	30
16 +	11	11	-----	43.8	38.4	-----	0.50	0.30	-----	0.44	0.10	-----	30	30	-----
17 +	15	15	-----	8.4	8.4	-----	1.11	0.65	-----	0.00	0.17	-----	45	45	-----
18 +	24	24	30	5.1	1.2	15.9	0.45	0.04	0.00	0.14	0.05	0.01	75	75	80
Means	16	18	18	7.0	7.7	18.0	0.75	0.58	0.03	0.22	0.31	0.02	46	46	38

[2]The live, streamside subplot data is an average of two live yew subplots.

Table 16, continued. **Elder Creek**

	Percent Slope			Slope distance to high water (m)			Basal area of dead POC			Basal area of live POC			Percent Canopy Cover		
Plot	dead	live-stream	live-uphill	dead	live-stream	live-uphill	dead	live-stream	live-uphill	dead	live-stream	live-uphill	dead	live-stream	live-uphill
28 +	24	50	48	4.8	1.8	15.3	0.03	0.71	0.00	0.10	0.05	0.01	2	2	2
29 -	21	17	40	3.6	3.6	12.0	1.19	1.21	0.00	0.17	0.12	0.01	75	75	85
30 +	27	5	40	0.6	10.7	12.0	1.55	1.17	0.00	0.47	0.11	0.05	75	75	85
31 +	37	36	23	1.8	3.7	14.4	0.60	0.73	0.00	0.46	0.19	0.00	55	55	35
32 +	42	34	-----	6.0	3.7	-----	1.17	1.27	-----	0.13	0.19	-----	50	50	-----
33 +	49	45	45	0.9	0.9	11.4	0.19	0.16	0.00	0.00	0.09	0.06	70	70	40
34 +	60	45	29	1.5	0.0	27.6	0.03	0.04	0.00	0.17	0.76	0.04	60	60	60
35 +	45	28	45	10.8	2.9	9.0	0.19	0.16	0.00	0.76	0.48	0.36	65	65	45
36 -	22	36	38	7.2	0.0	14.4	0.13	0.13	0.00	0.89	0.46	0.04	40	40	90
37 +	32	37	20	5.7	4.3	18.6	0.02	0.00	0.00	0.36	0.23	0.01	65	65	45
38 +	52	52	30	3.9	3.8	10.5	0.04	0.03	0.00	0.73	0.56	0.00	20	20	80
39 +	60	73	-----	0.0	4.7	-----	0.19	0.28	-----	0.34	0.05	-----	5	5	-----
40 +	18	32	40	0.0	3.0	14.4	0.59	0.58	0.00	0.04	0.00	0.07	50	40	80
41 +	33	55	-----	0.0	0.9	-----	0.02	0.00	-----	0.36	0.21	-----	15	15	-----
42 +	39	38	33	1.8	1.8	17.1	0.00	0.00	0.00	0.17	0.12	0.07	80	80	10
43 +	50	38	57	0.0	3.2	13.8	0.02	0.13	0.00	0.00	0.05	0.00	40	40	60
44 -	35	35	40	1.2	4.5	18.9	0.09	0.00	0.00	0.14	0.14	0.01	55	65	70
Means	38	39	37	2.9	3.1	14.9	0.35	0.39	0.00	0.31	0.22	0.05	48	48	56

Table 16, continued. **All other locations** (Plot 25 is Baker Flat, plots 2, 26 and 27 are Monkey Creek, and plots 45-47 are Panther Ridge.)

	Percent Slope			Slope distance to high water (m)			Basal area of dead POC			Basal area of live POC			Percent Canopy Cover		
Plot	**dead**	**live-stream**	**live-uphill**	**dead**	**live-stream**	**live-uphill**	**dead**	**live-stream**	**live-uphill**	**dead**	**live-stream**	**live-uphill**	**dead**	**live-stream**	**live-uphill**
2[2] +	55	58	74	0.6	0.0	13.2	1.04	0.61	0.00	0.02	0.07	0.01	30	42	10
25 +	15	15	7	0.0	3.9	8.7	0.25	0.16	0.00	0.04	0.07	0.00	90	90	85
26 +	64	87	-----	0.0	0.3	-----	0.03	0.03	-----	0.03	0.13	-----	50	50	-----
27 +	65	65	-----	0.0	0.0	-----	0.24	0.23	-----	0.13	0.00	-----	70	70	-----
45 +	15	6	15	0.0	4.2	9.9	0.08	0.00	0.00	0.38	0.17	0.01	50	45	10
46 +	5	5	50	0.0	0.3	12.9	0.16	0.04	0.00	0.19	0.09	0.00	20	15	10
47 +	4	4	40	0.0	1.8	11.7	0.03	0.03	0.00	0.05	0.02	0.05	50	75	70
Means	32	34	37	0.1	1.5	11.3	0.26	0.15	0.00	0.12	0.22	0.01	51	55	37

[2]The live, streamside subplot data is an average of two live yew subplots.

ELISA results for each dead subplot center tree are also shown in Table 16. ELISA was positive on 41 of the 47 trees included in this study. Baiting of *P. lateralis* was unsuccessful for all trees.

DISCUSSION

Mortality of Pacific yew was correlated positively with proximity to stream edges and with the interaction of stream proximity and shallowness of the slope. Roots of Pacific yew that occur on steep banks would be exposed to infested water at a lesser frequency and duration than roots of trees that are adjacent to the water, or level or nearly level with the water's edge. The results further suggest that the amount of dead POC in the immediate vicinity of diseased yews may be relatively unimportant in causing yew mortality, although the overall severity of the epidemic in the drainage likely influences the rate of yew mortality. Dead POC were located along the length of the study drainages, and were present at every dead and live stream-side subplot, although no difference in amount of cedar was detected between the two. Such evidence suggests that root contact with dead POC is not necessary, but that exposure to infested water is a more important factor in yew mortality. It should be noted, however, that a limitation of this study lay in the placement of the live stream-side pair. The live yew pair was between 9 and 15 m from its associated dead pair, and as a result, the basal areas of POC on some subplots overlapped.

In determining the basal area of the subplots as a measure of the amount of inoculum in the plot, it would have been desirable to begin with exact diameters of the POC trees. Unfortunately, at the time of data collection, the diameter classes were to be used for a different reason, and basal area was recorded with an angle gauge on a per hectare basis. It was later decided to record basal area on a per subplot basis, and it was necessary to modify the diameter class data that had been recorded. Basal area, however, may not have been a reliable measure of inoculum availability. It was used because it is easy to determine, and viewed no worse than ascertaining the number of *P. lateralis* propagules in soil, which has proven to be expensive and frequently unreliable.

All the uphill Pacific yew subplots were represented by live trees only. These plots were far from the water's edge, and the basal area of dead POC was minimal. Pacific yew growing in these areas probably have little chance of being killed by *P. lateralis*. Very little POC (live or dead) was recorded on the uphill plots, supporting the hypothesis that POC root rot is restricted primarily to the wetter stream-side locales. As a result, the Pacific yew growing uphill from the drainages are protected not only from exposure to infested water, but from localized inoculum build-up associated with killed POC.

Most of the dead yews included in this study occurred within 7 meters of the streams' high water marks. The dead yew tree at some plots, however, including plot 20 at Coon Creek, plot 16 at the Middle Fork, and plot 35 at Elder Creek, was greater than 10 meters from the stream's high water mark. Plot 20 was located in a seepage area, which accounts for the reason for its inclusion. Its ELISA result, however, was negative (Table 4a), suggesting that this tree could have died from means other than *P. lateralis*. Plot 16 at the Middle Fork was a dead yew tree previously identified by the USFS survey as positive for *P. lateralis*, and was also positive by ELISA. This tree was located near the far edge of a large floodplain with high POC mortality. Plot 35 at Elder Creek was located along an intermittent drainage that flowed into the main creek, and that had upstream POC mortality. The ELISA absorption reading for this dead yew was positive.

Although 6 of the 47 dead yew trees yielded negative ELISA readings, measurements of those trees remained in the study for several reasons. First, those dead trees were located adjacent to, or very close to the water's edge, suggesting a cause of death attributable to *P. lateralis*. Second, although the ELISA test is relatively sensitive, *Phytophthora* must be present in the tissue for positive readings, and only a relatively small portion of the dead trees was sampled. Unfortunately, due to time constraints, a second sample could not be extracted from the tree to confirm or deny the results via a second ELISA test. Finally, aside from drought, no other causative agent of Pacific yew mortality in areas such as those surveyed in this study is known to exist.

Diagnosis of tree death can be a time-consuming and often frustrating procedure. Baiting for *P. lateralis* has been used in the past because it is a fungal-specific technique that provides consistent results. Older tissue and older samples can be used, although results are not available for at least 9 days. Detection of *P. lateralis* via baiting was unsuccessful for all of the dead yew subplot trees in this study, even though they possessed the symptomotology characteristic of infection by this pathogen, including phloem discoloration and foliar wilting. When those same samples were used in the ELISA test, however, a majority of the results were positive. *Phytophthora* must be viable in the tissue for baiting to work, while only remnants of the pathogen are necessary for detection with ELISA. These results suggest a low survival rate of *P. lateralis* in Pacific yew tissue once the tree is dead. Maximum survival of the fungus in POC tissue has been estimated at 7 years (Hansen, Hamm, in preparation).

The ELISA test proved to be an efficient means of detecting *P. lateralis* in Pacific yew tissue. Although Neogen's ELISA test is sensitive to most species of *Phytophthora* including *P. cinnamomi* Rands., and slightly sensitive to some *Pythium* spp., the authors are confident that the positive readings were a result of *P. lateralis* only. Technical handouts provided by the company list the specificity of the Phytophthora ELISA Kit: "The Phytophthora Kit is highly specific for *Phytophthora* spp. and does not react with other genera of fungi including....most species of *Pythium*. Pure culture extracts of *Pythium coloratum* and *P. vexans* show significant cross reactivity in this assay. *Pythium aphanidermatum*, *P. dissotocum*, and *P. ultimum* may show low levels of reactivity in the assay."

Phytophthora cinnamomi has been reported on *Taxus* species in nurseries (Schreiber and Green 1959) as well as on POC ornamentals (Torgeson 1952). However, the possibility of *P. cinnamomi* contributing to the mortality of either host species in forested situations has been disputed by Hansen, Hamm, and Roth (1989). The authors made many isolations from soil, water, and infected plant material in forested areas, and recovered no *Phytophthora* species other than *P. lateralis*. Unlike *P. cinnamomi*, *P. lateralis* is adapted to Pacific Northwest's cool, wet winters and hot, dry summers. And if a *Pythium* species contributed to the positive readings in dead and dying trees, then the results of the dead yew that had died due to drought, and dead controls in previous studies (Hansen, pers. comm.) may have been positive as well.

Some degree of Pacific yew mortality can be expected in areas where this species occurs in close association with slow moving, *P. lateralis*-infested water in the native range of POC. In the Coast Range and Klamath Mountains of northern California, Pacific yew is most commonly associated with the cool, wet riparian areas in the POC series and least commonly associated at mid to upper slopes and/or in the Douglas-fir or white fir/red fir series (Scher and Jimerson 1989). Hence, the amount of reported mortality in both the 1991 USFS survey and the field survey discussed in Chapter 2 was greatest in northern California. In those areas, Pacific yew provides wildlife cover and food, and contributes to stream channel stabilization through its fibrous root system. If mortality is high in areas dominated by Pacific yew, ecological consequences may include stream channel erosion, a local increase in stream temperature, and loss of general habitat for certain wildlife species.

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Chapter 4. Conclusions

This study was the first examination of the susceptibility of Pacific yew to *Phytophthora lateralis*. In the past, little focus was placed on the yew tree due to its lack of value as a timber species, although its ecosystem value ranges from stream channel stabilization to wildlife habitat. Once it was reported as a host for *P. lateralis*, managers began to question the role of Pacific yew in survival and spread of the fungus in harvest sites and in areas containing Port-Orford-cedar (POC). The goal of our study was to show that Pacific yew is a less susceptible host than POC, but that it can be killed in areas of regular soil saturation and high inoculum. The following conclusions summarize our results.

1. Mortality, root necrosis, and lesion length were significantly higher in the POC seedlings than in the Pacific yew seedlings and cuttings, as determined by four different root inoculation methods. In addition, *P. lateralis* grew faster in POC branches and stems than in comparable Pacific yew tissues.

2. The root dip method of inoculation resulted in the greatest mortality and damage of Pacific yew seedlings and cuttings. Inoculating the soil with chlamydospore, planting yews alongside an infected POC seedling, and planting yews in soil amended with infected POC rootlets resulted less consistent disease development.

3. Percent necrotic root tips was not an accurate measurement of susceptibility because non-infected control seedlings and cuttings also expressed this symptom.

4. Results of Pacific yew root, stem, and branch inoculations suggest that this species may vary in susceptibility to *P. lateralis*. This variation also may be attributable to other factors including type and amount of inoculum; age, type, and geographic source of material; or a difference in environmental conditions between inoculations.

5. Growth of *P. lateralis* in POC roots, stems, and branches was not strongly influenced by the source or type of plant material.

6. Compared to POC, significantly fewer zoospores encysted on the Pacific yew root cap, the zone of elongation, and the cut end.

7. On POC rootlets, the greatest number of zoospores encysted along the zone of elongation, while on Pacific yew rootlets, most zoospores encysted on root hairs along the region of maturation.

8. *Phytophthora lateralis*-induced mortality in the field is far greater in POC than in Pacific yew.

9. The extent of mortality of Pacific yew in the field was greater than previously reported, but unlikely to significantly threaten the species.

10. ELISA is an effective means for detecting *P. lateralis* in Pacific yew tissue.

11. At forest sites surveyed, mortality of Pacific yew occurred most often on sites adjacent to or close to *P. lateralis*-infested streams, where the slope of the land in the vicinity of the stream was very shallow.

The management policy for POC is based upon the Interregional Port-Orford-cedar Action Plan (Barker and Torrence 1988), drafted as a commitment by members of the U.S. Forest Service in Regions Five and Six to ensure the continued presence of POC in its native ecosystem. Guidelines were established for various management activities including engineering and road building, timber harvest, and stand management, with the main objectives being prevention of spread into uninfested areas and out of infested areas, and reducing the rate of spread within infested areas. The Port-Orford-cedar Management Guide states that, in areas where Pacific yew and POC occur together, Pacific yew will be analyzed for risk to infection, but no mitigation measures were established in management areas where Pacific yew occurs alone (Greenup 1994). The interim guide to the conservation and management of Pacific yew, written at the time when yew was being harvested for taxol, stresses that harvest of yew in areas where POC occurs should be implemented with control strategies in mind, and in areas outside the range of POC, the potential for introduction of the disease should be evaluated (Berry 1992).

Results of this study support the current management policy for areas where Pacific yew is not associated with POC. For initial infection of Pacific yew, the amount of *P. lateralis* in the area must be relatively high, which likely occurs when the more susceptible POC is present. Port-Orford-cedar is associated with wet areas, and in drainages, this species is abundant, resulting in ideal conditions for inoculum to increase. Infection of yew may occur in sites without POC, but mortality, if it occurs, will be slow.

The USFS previously reported the presence of 17 dead Pacific yew trees due to infection by *P. lateralis*. Results of this study show that at least 5 times that many trees, or 10 percent of the population surveyed, are dead along just three, 0.8-km-stretches of infested streams. The presence of *P. lateralis* was confirmed in 41 of those trees. Not all infested locations in the range of POC were included in either survey, so the amount of dead Pacific yew is undoubtedly higher.

Compared to POC, however, mortality of yew is currently quite low, but managers should not be deceived by the low mortality rate in these infested drainages. *Phytophthora lateralis* grows very slowly on Pacific yew, and trees may be infected without showing above-ground symptoms, which could increase the chance of infection on surviving POC if the fungus is sporulating from the yew roots. It is doubtful that the presence of absence of yew in a drainage contributes significantly to mortality of established POC, but yew could provide a refuge for survival of *P. lateralis*. In areas where eradication of *P. lateralis* by removal of POC is desired, managers should consider removing all live Pacific yew as well.

The origin of *Phytophthora lateralis* in the Pacific Northwest has long been a subject of discussion among scientists and others. Due to the epiphytology of the pathogen on POC and the fact that the first reports of dying cedar originated in port cities, most believe the fungus to be introduced. Some have suggested Asia as an origin because Asian *Chamaecyparis* species are more resistant to infection. Others speculate that *P. lateralis* is endemic to Alaska yellow-cedar, another tolerant species, in southeast Alaska and northwest British Columbia.

To this speculation, we can now add Pacific yew, native to the Pacific Northwest, as the possible original host of the fungus. *Phytophthora lateralis* was first introduced into the northern-most portion of the native range of POC in 1952, where it was progressively spread south and inland, as indicated by the wake of cedar mortality. Since Pacific yew was never a commodity species, mortality was not a concern, so time of original mortality in that area is unknown. Infection of Pacific yew was first reported in 1991 (DeNitto and Kliejunas), however, mortality probably occurred long before this time. A total of 86 dead yew trees were tallied in the stream survey, and almost half had missing bark and white-bleached wood, suggesting a time since death of more than 10 years.

Pacific yew is a wide-ranging species, and occurs from the southern tip of southeast Alaska south to northern California, and west to the northern Rocky Mountains in British Columbia, Idaho, and Montana. Survival of *P. lateralis* on yew could have occurred for centuries, and with the increase in forest management and transportation in the western states in the early 1900's, movement of the pathogen could have progressively occurred until it was reported on POC near Seattle, WA, in the early 20's.

Additionally, greenhouse inoculated Pacific yew are able to withstand mortality in the presence of *P. lateralis* while maintaining the pathogen. Survival of the fungus was detected in roots of asymptomatic seedlings 20 wk after inoculation in preliminary experiments, and 15 wk after inoculation in the experiments reported in this study. Mortality was less than 5 percent, and growth of the pathogen in the tissue was slow as compared to growth in its primary host, POC. The fact that Pacific yew is partially resistant to *P. lateralis* except under extreme circumstances, suggests that the host and pathogen have evolved together.

The role of taxol, one possible defense mechanism, was recently examined in *Taxus* species (Wagner and Flores 1994; Elmer, Mattina, and MacEachern 1994). Researchers found that in the presence of taxol and cephalomannine, growth of several plant pathogenic fungi, especially those in the Oomycetes, is reduced. Wagner and Flores looked specifically at growth of *P. lateralis* in liquid and on solid media, and found that taxol and cephalomannine significantly reduced colony growth. Taxol production in several *Taxus* species, however, is greatest in the bark tissue, and least in the root tissue (Vidensek, et al. 1990) where the pathogen occurs, although comparisons have not been made using *Taxus brevifolia*.

A small, widely scattered population of Pacific yew has succumbed to the pathogen in infested drainages in POC's native range. Results of this study suggest that mortality of yew in the field is more likely to occur in areas with seasonally saturated soils and on gentle slopes, while results of greenhouse inoculations suggest that a variation in susceptibility exists among individuals. If taxol does play a role in host resistance, then, along with environmental conditions, tree-specific variation in taxol production could explain a small part of the variation in susceptibility. Wheeler, et al. (1992) found that taxol content varied among individuals within and between populations. Trees growing in cool, moist sites in Washington state had the highest average taxol content, while trees growing in warm, dry areas, including those in southern Oregon, had some of the lowest taxol contents. The authors suggest that this variation is due to environmental factors, and not genetic heritability. Other factors involved in yew mortality may include excessive root wounding or tree damage. Mortality and root necrosis were greatest in the seedlings and cuttings whose roots had been cut prior to exposure to *P. lateralis*.

Several areas of research have yet to be addressed, including those raised by the results of this study. The following list summarizes some important questions to examine.

1. What is the mechanism by which *P. lateralis* infects and colonizes Pacific yew root tissue?

2. Are the roots of Pacific yew killed by *P. lateralis* in the field excessively damaged?

3. What percent of live trees in infested drainages are infected with *P. lateralis*?

4. What is the survival rate of *P. lateralis* in Pacific yew tissue once the tree dies?

5. Are Pacific yew trees that occur in the range of POC more susceptible to *P. lateralis* than trees outside the range?

6. Does *P. lateralis* occur on Pacific yew outside the native range of POC?

7. Does the production of taxol in needles, bark, and/or roots of Pacific yew growing in infested drainages, especially those with the potential for mortality (occurring in or adjacent to the streambed) change over time?

8. Does the production of taxol in Pacific yew needles, bark and/or roots increase after infection by *P. lateralis*?

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